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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

**MEMORANDUM**

Date: 21-August-2003

Subject: ID# 0F06210 - Section 3 Registration for Application of Glufosinate Ammonium to Transgenic Rice. **HED Response to Sierra Club Comments Concerning the Notice of Filing for Application of Glufosinate Ammonium to Transgenic Rice.** DP Barcode D292894. Chemical 128850. Case 293386. Submission S635308.

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To: Joanne Miller/James Stone, PM Team 23
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Neil J. Carman, Ph.D., of the Sierra Club Genetic Engineering Committee, submitted comments concerning the Notice of Filing (NOF) associated with the petition for application of glufosinate ammonium to transgenic rice (letter dated 23-Aug-2002). The RD has requested that HED draft a response to these comments. The following document is in response to RD's request.

HED notes that the NOF represents a summary of the petition prepared by the petitioner and represents the view of the petitioner (see section II of the NOF). As such, and in this case, discrepancies may arise between what is stated in the NOF and the procedures/conclusions employed by HED when assessing human health risk. For instance, the toxicological database for glufosinate ammonium has been reevaluated by HED since August, 2002, and some of the conclusions presented in the NOF concerning the toxicity of glufosinate ammonium do not reflect conclusion made by HED (see HED document TXR 0051833).

***Sierra Club Comment A(1) - Plant Metabolism of Glufosinate.*** A concern is other plant metabolites of glufosinate-ammonium may occur in addition to the two primary metabolites identified by Aventis in the grain and straw, since the two substances did not appear to account for 100% of the total radioactive residues in the two plant tissues tested. While more than 80% appeared to be accounted for, Aventis needs to identify whether additional metabolites were produced. The two primary metabolites identified as being typical of plant metabolism in the grain at harvest were 3-methylphosphinicopropionic acid, being -70% of the total radioactive residues (TRR). Another residue in the grain was N-acetyl-L-glufosinate (2-acetamido-4-methylphosphinicobutanoic acid), at about 11% of the TRR and parent at 5-6% of the TRR. In the straw, 3-methylphosphinicopropionic acid was the major metabolite comprising approximately 60% of the TRR. Lesser amounts of the parent (about 17% of the TRR) and N-acetylglufosinate (10-13% of TRR) were found in the straw fraction.

*HED Response:* The transgenic rice metabolism study was conducted according to the regulatory guideline requirements (OPPTS 860.1500) and conformed to EPA Good Laboratory Practice (GLP) Standards (the % TRR figures given below are averages of 4 samples). The study indicated that glufosinate ammonium, N-acetyl-glufosinate, and 3-MP accounted for 88% and 91% of the total radioactive residue (TRR) found in rice grain and rice straw, respectively (grain and straw are the only rice raw agricultural commodities (RACs)). The remainder of the radioactivity was identified as 2-methylphosphinico-acetic acid (grain - 1% TRR; straw - 2% TRR), several unknowns when combined accounted for 2% TRR (rice grain) and 3% TRR (rice straw), and fiber bound residues (grain - 8% TRR; straw - 5% TRR). The petitioner identified/characterized 99% and 101% of the TRR in rice grain and rice straw, respectively. In previously submitted transgenic canola (D257628, T. Bloem, 9-Jul-1999) and nontransgenic apple, corn, lettuce, soybeans, and wheat (PP# 8F3607, J. Garbus, 14-Oct-1988 and 8-Aug-1990) metabolism studies, the petitioner demonstrated the incorporation of radioactivity into nature plant constituents. On the basis of the transgenic rice metabolism study and the previously submitted metabolism studies, HED concluded that the residue identification/characterization procedures performed were adequate and the residues of concern in transgenic rice, for purposes of tolerance enforcement and risk assessment, are glufosinate ammonium, N-acetyl-glufosinate, and 3-methylphosphinico-propionic acid (3-MP).

***Sierra Club Comment A(2) - Analytical method.*** We ask EPA if any independent sampling and gas chromatography analyses were conducted besides that performed by Aventis and its contractors. We request that an independent sampling and G.C. analysis program be carried out if Aventis has not had a third party independent contractor, since we are skeptical of Aventis' sampling data and analyses. We generally agree that the enforcement analytical method of utilizing gas chromatography appears to be acceptable for detecting and measuring levels of glufosinate-ammonium and metabolites with a general limit of quantification of 0.05 ppm to allow detection of glufosinate residues at or above the proposed tolerances. We wonder if glufosinate residues might have been found between 0.01 ppm and 0.05 ppm, and that due to its toxicity, EPA should have required a lower detectability limit be utilized to demonstrate if glufosinate residues were missed below 0.05 ppm or 50 parts per billion (ppb) concentration down to 1 ppb.

*HED Response:* The rice magnitude of the residue study was conducted according to the regulatory guideline requirements (OPPTS 860.1500) and conformed to EPA GLP Standards. The rice grain and straw samples were analyzed using a method similar to that previously validated by an independent laboratory and by the EPA. Based on these validation procedures and the validation and concurrent recovery data submitted with the transgenic rice field trials, HED concluded that the method was appropriate for data collection purposes.

HED understand that residues <LOQ does not mean that residues are not present. The dietary analyses assumed average field trial residues for rice commodities. When calculating the average, half LOQ residues were assumed for residues <LOQ. Therefore, the dietary risk assessment took into account the possibility of residues between 0.01 and 0.05 ppm. For further information on HED's rationale for assuming half LOQ residues see "Values to Non-Detectable/Non-Quantifiable Residues in Human Health Food Exposure Assessments" (faxed upon request; telephone: (202) 401-0527; item: 6047).

***Sierra Club Comment A(3) - Magnitude of Glufosinate Residues.*** The reason that we are requesting independent sampling and gas chromatography analyses be conducted besides that performed by Aventis and its contractors is the potential for higher glufosinate residue concentrations to be confirmed above the 0.74 ppm level in rice grain and 1.48 ppm level in rice straw when sampled at 70 days or more after the last treatment. We are concerned that Aventis' sampling protocol may have been biased in some unidentified manner and that samples above the 0.74 ppm level in rice grain and 1.48 ppm level in rice straw were missed in the field residue trials. While EPA emphasizes that the treatment regime was selected to represent the use pattern that is the most likely to result in the highest residues, we are concerned that sampling bias may have transpired and resulted in bias in the G.C. analyses. We are also concerned that glufosinate treatment may have occurred closer to the sampling period than is the case and higher glufosinate concentrations were missed. After all a higher concentration factor of approximately 2.3 was found for rice hulls compared to the grain and straw. We also question that the finding that no detectable concentration of the residues occurred when rice whole grain was processed into polished grain and bran, whereas a glufosinate residue concentrations may have been present at less than the 0.05 ppm (50 ppb) detection limit.

*HED Response:* The rice magnitude of the residue (15 field trials conducted throughout the rice growing regions in the US; two composite samples collected at each site) and processing studies were conducted according to the regulatory guideline requirements (OPPTS 860.1500 and 860.1520) and conformed to EPA GLP Standards. It is difficult to further address the potential for bias since the comment gave no specific criteria for the concern. The comment does make reference to the processing study and the concentration of residues in rice hull and the lack of concentration of residues in rice bran and polished rice. The following paragraph is a summary of the rice processing study.

Processing studies are required to determine if residues reduce or concentrate during food processing (processing factor = concentration in processed commodity ÷ concentration in unprocessed commodity). Processing factors are dependent on several factors including the location of the residues (surface or translocated residues), loss of water as in dried commodities, and/or the physical chemical properties of the residues. The rice processing study (conducted at 5x the proposed rate) resulted in quantifiable concentrations of glufosinate ammonium, N-acetyl-

glufosinate, and 3-MP in/on all commodities excluding glufosinate ammonium and N-acetyl-glufosinate in/on rice hull (residues at the LOQ assumed for calculation of processing factor). Based on the combined glufosinate ammonium, N-acetyl-glufosinate, and 3-MP residues in/on the processed and unprocessed commodities, the following processing factors were calculated: rice hull - 2.8x, rice bran - 0.9x, and polished rice - 1.3x. The dietary analyses assumed average field trial residues and a processing factor of 1.3 for all rice commodities excluding rice bran where a processing factor of 0.9 was assumed (rice hull is not a human food commodity).

***Sierra Club Comment B(1) - Acute Toxicity.*** EPA states that glufosinate ammonium has been classified as toxicity category III for acute oral, dermal, and inhalation toxicity; and for eye irritation. EPA finds that glufosinate-ammonium is not a dermal irritant (toxicity category IV) nor is it a dermal sensitizer. The oral LD50 is 2 g/kg in male rats, and 1.62 g/kg in female rats. But we are concerned about acute toxicity because of the published finding that glufosinate causes convulsions in humans and experimental rodents by brain cell glutamate receptor activation (glufosinate and glutamate are structurally similar) according to Matsumura et al (1). Has EPA considered the structural similarities between glufosinate and glutamate receptor activation. We request that EPA review all of the relevant toxicological literature on human and rat brain cell glutamate receptor activation and speak with scientists who performed this research as to the significance of glufosinate tampering with glutamate receptors.

Evidence also exists that glufosinate stimulates nitric oxide production in the brain through N-methyDaspartate (NMDA) receptors (2). We request that EPA investigate this published finding to determine if the requested herbicide tolerance concentrations are set too high which is a possibility.

*HED Response:* EPA has evaluated both the published and petitioner submitted toxicity studies. The oral, dermal, and inhalation toxicity categories assigned by EPA are based on studies conducted according to the EPA toxicity testing guidelines and were conducted in compliance with EPA GLP. In an acute oral toxicity study in rats, the oral $LD_{50}$ was found to be 1620 and 2000 mg/kg/day in female and male rats, respectively. In this study, no effects were seen in rats at doses up to 630 mg/kg/day.

The commenter cites two acute exposure studies. Matsumura *et al.* have shown that an acute dose of 80 mg/kg injected intraperitoneally into mice was convulsive and that this effect was partially antagonized by NMDA antagonists, suggesting that NMDA receptors may mediate this effect. Nakaki *et al.* found that injection of glufosinate ammonium directly into the brain stimulated nitric oxide production as a result of stimulation of NMDA receptors in rat brain, another neurochemical effect. But neither of these published studies provide sufficient appropriate evidence to establish an acute endpoint for risk assessment from oral, dermal, or inhalation exposures because the routes that they used, intraperitoneal injection or direct injection into the brain, are not directly relevant to potential routes of human exposure to pesticides, i.e., oral, dermal, or inhalation exposure.

The herbicidal mechanism of action of glufosinate-ammonium is inhibition of the enzyme glutamine synthetase. This enzyme is also present in mammalian systems. In mammals, glutamine synthetase facilitates the conversion of glutamate and ammonia to glutamine and is therefore involved in the metabolism of nitrogen and ammonia. In addition, glutamate is a major

excitatory neurotransmitter in the nervous system; inhibition of glutamine synthetase has been shown to impair its ability to serve as a neuroprotectant by controlling glutamate concentrations in neurons. More generally in the body, ammonia is buffered for extracellular transport through its interaction with glutamate to form glutamine by glutamine synthetase.

EPA also reviewed mechanistic studies submitted by the petitioner as well as the published studies, and, where applicable and appropriate, incorporated findings from these studies in the human health risk assessment. In fact, the intermediate-term and long-term incidental oral endpoints and the chronic dietary endpoint are based on brain glutamine synthetase inhibition, the most sensitive indicator of glufosinate ammonium toxicity in humans and experimental animals.

Tolerances are not risk based standards. EPA establishes maximum residue limits, or "tolerances," for pesticide residues in food under section 408 of the FFDCA. 21 U.S.C. § 346a. Without such a tolerance or an exemption from the requirement of a tolerance, a food containing a pesticide residue is "adulterated" under section 402 of the FFDCA and may not be legally moved in interstate commerce. 21 U.S.C. § 331, 342. Monitoring and enforcement of pesticide tolerances are carried out by the U.S. Food and Drug Administration (FDA) and the U. S. Department of Agriculture (USDA).

***Sierra Club Comment B(2) - Genotoxicity.*** EPA claims that ... based on results of a complete genotoxicity database, there is no evidence of mutagenic activity in a battery of studies, including: Salmonella spp., E. coli, in vitro mammalian cell gene mutation assays, mammalian cell chromosome aberration assays, in vivo mouse bone marrow micronucleus assays, and unscheduled DNA synthesis assays. EPA needs to inquire with the FDA, USDA, ATSDR, medical doctors and scientists as to whether there are reports of glufosinate-induced mutations and gene toxicity which appear to be glossed over in the Aventis petition.

*HED Response:* Glufosinate ammonium was clearly negative in the acceptable guideline mutagenicity studies. The test battery included: a *Salmonella typhimurium* and *Escherichia coli* reverse gene mutation assay, *in vitro* mammalian cell gene mutation and chromosome aberration assays, *in vivo* mouse bone marrow micronucleus assay and an *in* vitro unscheduled DNA synthesis assay. All studies were performed in accordance with the specified Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Harmonized Mutagenicity Test Guidelines Series 870 and satisfied the testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Toxic Substances Control Act (TSCA), and the Organization for Economic Cooperation and Development (OECD). Further, each study meets the requirement of 40 CFR Part 160, Good Laboratory Practice (GLP) and was subjected to a Quality Assurance(QA) inspection. Based on the negative responses observed in these assays, EPA concluded that there is no concern for mutagenicity from exposure to glufosinate ammonium (TXR No. 0051833). In addition, no evidence of carcinogenicity was observed in mice and rats in acceptable guideline carcinogenicity studies. As indicated previously, EPA evaluated both petitioner submitted guideline studies and published scientific studies. In addition, the petitioner is required by law under FIFRA (6(a)2) to report any adverse finding to EPA.

No mutagenicity studies were found in the open literature and the Agency for Toxic Substances and Disease Registry (ATSDR) has no finalized, draft or "under development" Toxicological Profile for glufosinate ammonium. Finally, FDA has evaluated the human safety of multiple

crops with resistance to glufosinate ammonium and has no concerns for human safety but has no mutagenicity or toxicity data in the Biotechnology Notification Files on this herbicide.

***Sierra Club Comment B(3) - Reproductive and Developmental Toxicity.*** We are skeptical of EPA's findings because, based on peer-reviewed studies in the published literature, birth defects have been caused by exposure of the human father to the herbicide (3). EPA needs to thoroughly investigate these findings and reconsider the glufosinate herbicide tolerance limits requested by Aventis as entirely unsafe and unacceptably high.

It is rather distressing to note that there do not seem to be peer reviewed studies on the metabolism of the high levels of acetyl glufosinate in harvested GM crops to highly neurotoxic and teratogenic glufosinate. Certainly gut bacteria are well known to contain enzymes that remove acetyl groups from glufosinate and mammalian enzymes may also be capable of removing the acetyl group from glufosinate. Even though glufosinate is being used widely with GM crops in North America its safety is far from proven and its impact on humans and farm animals is difficult to trace because the GM products are not labeled for consumption. We request that EPA obtain more technical data and information to better define the neurotoxicity and teratogenicity of glufosinate and its metabolites, especially in humans.

Glufosinate, for example, was found to trigger apoptosis (programmed cell suicide) in the developing brain of the embryonic mouse (5). Numerous well established studies showing brain damage and birth defects seem to have been ignored by those regulating use of the herbicide. We request that the EPA conduct a more comprehensive investigation of available literature on glufosinate and make requests for unpublished information from independent scientists such as their expert opinions on the adverse health effects of glufosinate and its metabolites.

We request the same under subchronic (Sierra Club Comment B(4)), chronic (Sierra Club Comment B(5)), animal metabolism (Sierra Club Comment B(6)), and metabolite toxicology (Sierra Club Comment B(7)) as requested for Reproductive and Developmental toxicity.

*HED Response:* The study authors (cited study by Garcia et al) state in their conclusion that "these findings warrant further investigation." In this study, only 16 individuals out of 261 referenced glufosinate ammonium. The results of this study indicated that there was a marginally significant increased risk of paternally related developmental toxicity. However, in this study various contributing factors such as smoking, work habits etc. were not evaluated and therefore, this epidemiological evaluation does not establish a causal definitive link to paternally related developmental toxicity. The potential for glufosinate ammonium to cause developmental or reproductive effects due to exposure (male or female) has been evaluated in acceptable guideline studies in rats and rabbits. Based on these studies, glufosinate ammonium is not teratogenic in rats and rabbits.

The petitioner has submitted acute, subchronic, chronic, developmental, and reproductive toxicity studies conducted with glufosinate ammonium. The petitioner has also submitted developmental toxicity studies (rat and rabbit) and subchronic studies (rat, mouse, and dog) with N-acetyl-glufosinate and 3-MP. All of these studies were conducted according to the regulatory guideline requirements (OPPTS 870 series) and conformed to EPA GLP Standards. HED has reviewed all of these studies and selected the most sensitive endpoints (see TXR No. 0051833 for further

information). Based on a comparison of the common studies conducted with the parent and metabolites, the metabolites exhibited toxic effects at doses equal to or greater than the parent and HED concluded that N-acetyl-glufosinate and 3-MP are not likely to be more toxic than glufosinate ammonium (risk assessment assumes they are of equal toxicity to parent; see D282757, 9-May-2002 memorandum). In regards to the enzyme that can remove acetyl groups from substrates, these enzymes are present in the toxicology test systems used to evaluate the parent and metabolites.

In the cited study by Watanabe, mouse embryo cultures were exposed to glufosinate ammonium. This is an *in vitro* experiments which indicate apoptosis in the developing brain of cultured mouse embryos. It should be noted that apoptosis is a normal part of the brain development process. This experiments did not use whole animals and the current scientific knowledge is not sufficient to allow extrapolation of *in vitro* results to whole animals.

***Sierra Club Comment B(8) - Endocrine disruption.*** We find EPA's statements on the potential of glufosinate to function as an endocrine-disrupting substance in humans and animals as not founded on logical information or peer-reviewed studies. In fact EPA states that no special studies have been conducted to investigate the potential of glufosinate-ammonium to induce estrogenic or other endocrine effects. Given the enormous complexities of mammalian hormonal regulatory systems and the current uncertainties existing in this field of knowledge as revealed by EPA's Endocrine Disruptor Advisory Committee several years ago about how to screen for potential endocrine-disrupting substances, we feel it's totally premature for EPA at this time to dismiss all concerns about glufosinate as an endocrine-disrupting substance. EPA stresses that no evidence of estrogenic or other endocrine effects have been noted in [[Page 48468]] any of the toxicology studies that have been conducted with this product and there is no reason to suspect that any such effects would be likely. Due to the millions of Americans and their children exposed to glufosinate and its metabolites, EPA needs to conclusively determine if this herbicide has endocrine-disrupting potential.

*HED Response:* EPA is required under the Federal Food Drug and Cosmetic Act (FFDCA), as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific bases for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA has authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

When the appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, glufosinate ammonium may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption. The studies submitted

as guideline studies as well as the data reviewed in the open literature did not provide any obvious indications that glufosinate ammonium and/or its metabolites have specific endocrine disruptive effects.

***Sierra Club Comment C(1) - Dietary exposure.*** EPA states that tolerances have been established (40 CFR 180.473) for the combined residues of glufosinate-ammonium and metabolites in or on a variety of RACs. EPA further maintains that no appropriate toxicological endpoint attributable to a single exposure was identified in the available toxicity studies. This is why EPA has not established an acute RfD for the general population including infants and children. An acute RfD of 0.063 mg/kg/day was established, however, for the females 13+ subgroup. Therefore, an acute dietary analysis was conducted for this sub-population; whereas, chronic dietary analysis was conducted for the usual populations. We request that EPA reconsider and reevaluate the health information finding that no appropriate toxicological endpoint attributable to a single exposure was identified in the available toxicity studies as too being limited and erroneous.

*HED Response:* EPA has evaluated the published toxicity studies and considered the relevant petitioner submitted studies. On the basis of these studies, no appropriate endpoint of concern attributable to a single exposure was identified. EPA has asked the petitioner to conduct a study to evaluate potential effects of glufosinate ammonium following a single exposure (acute effects) with glutamate synthetase measurements. Until such data are available, EPA has applied additional database UF to account or allow for uncertainty about those potential effects of acute exposure.

***Sierra Club Comment C(2) - Infants and Children.*** We are very concerned that EPA finds that the toxicological database is sufficient for evaluating prenatal and postnatal toxicity for glufosinate-ammonium in human infants and children using exclusively results from rats and rabbits. Although EPA states that there are no prenatal or postnatal susceptibility concerns for infants and children, based on the results of the rat and rabbit developmental toxicity studies and the 2-generation reproduction study, we are concerned that human infants and children may possess genetic predispositions, biochemical individualities and behavioral patterns very different from rats and rabbits. EPA needs to do a more thorough literature review and interview scientists and medical doctors who may have relevant information on the prenatal and postnatal toxicity for glufosinate-ammonium in human infants and children.

As EPA notes, Based on clinical signs of neurological toxicity in short [[Page 48469]] and intermediate dermal toxicity studies with rats, the agency has determined that an added FQPA safety factor of 3x is appropriate of assessing the risk of glufosinate-ammonium derived residues in crop commodities. Using the conservative assumptions described in the exposure section above, the percent of the chronic RfD that will be used for exposure to residues of glufosinate-ammonium in food for children 1-6 (the most highly exposed sub-group) is 61%. Infants utilize 37% of the chronic RfD. As in the adult situation, drinking water levels of comparison are higher than the worst case DWECs and are expected to use well below 100% of the RfD, if they occur at all. Therefore, there is a reasonable certainty that no harm will occur to infants and children from aggregate exposure to residues of glufosinate-ammonium.

*HED Response:* The short-term (dermal, inhalation, and incidental oral) and acute dietary (females 13-50 years) endpoints are based on reduced fetal body weight and increased fetal death seen in

the rabbit developmental toxicity study (6.3 mg/kg/day). An acute dietary endpoint for the general population, including infants and children, could not be identified due to no adverse effects seen in the relevant studies. The chronic dietary endpoint is based on a weight-of-evidence approach from several studies which demonstrated brain glutamine synthetase inhibition and alterations in the electrocardiogram (6.0 mg/kg/day). HED concluded that the toxicological database for glufosinate ammonium was not complete and requested the submission of the following studies: (1) acute neurotoxicity study conducted in the rat which includes glutamine synthetase activity measurement in the liver, kidneys, and brain; (2) a developmental neurotoxicity (DNT) study conducted in the rat which includes comparative glutamine synthetase activity measurement in the liver, kidneys, and brain of the pups and mothers; and (3) a 28-day inhalation toxicity study in rats with glutamine synthetase activity measurements in brain, kidney, liver and lung. HED also requested additional data to confirm that liver and kidney changes, observed in the absence of histopathological changes, are an adaptive response and not an adverse effect. Kidney and liver function assays should be performed in addition to glutamine synthetase activity measurements (HED document TXR 0051833). Pending the submission of the requested data, a 10x database uncertainty factor was applied to all oral and dermal risk assessments and a 100x uncertainty factor was applied to all inhalation risk assessments. These uncertainty factors combined with the traditional 100x inter/intra species uncertainty factor, resulted in a total uncertainty factor of 1000x for dermal and oral exposure assessments and 3000x for inhalation exposure assessments (10,000x uncertainty factor reduced to 3000x based on Agency policy, EPA/630/P-02/022F, December 2002).

The HIARC concluded that there is no qualitative or quantitative evidence of increased susceptibility in the developmental toxicity study conducted in rats. Qualitative evidence of increased susceptibility is demonstrated in the rabbit developmental toxicity study since fetal deaths were observed in the presence of lesser maternal toxicity at the same dose. There is also quantitative evidence of increased susceptibility in the rat 2-generation reproduction study. In this study, a decrease in the number of viable pups was observed in the absence of parental toxicity at any dose. Since there is qualitative evidence of increased susceptibility of the young following exposure to glufosinate ammonium, HIARC performed a degree of concern analysis to: (1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and (2) identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of this chemical. Based on the data gaps listed above, the HIARC did not identify any other residual uncertainties. The established endpoints are protective of pre-pre/postnatal toxicity following acute and chronic exposures.

***Sierra Club Comment D - Cumulative Effects Section 408(b)(2)(D)(v).*** We are deeply concerned about the potential for cumulative effects of glufosinate and its metabolites, and therefore request that EPA not approve the Aventis tolerance petition unless or until peer-reviewed confirming scientific evidence is available that glufosinate and its metabolites do not cause any cumulative effects. It is not acceptable public health policy to dismiss cumulative effects of glufosinate and its metabolites because of lack of scientific evidence and lack if any studies. Law requires that, when considering whether to establish, modify, or revoke a tolerance, the EPA must consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity." EPA has indicated that, at this time, the Agency does not have available data to determine whether glufosinate-ammonium has a

common mechanism of toxicity with other substances or how to include this pesticide in a cumulative risk assessment. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA suggests that glufosinate-ammonium does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance petition, therefore, it has not been assumed that glufosinate-ammonium has a common mechanism of toxicity with other substances. We disagree with EPA's illogical and unscientific assumption that glufosinate-ammonium has a common mechanism of toxicity with other substances. We propose that further study is necessary to conclusively confirm such an assumption.

*HED Response:* Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA does not have, at this time, sufficient data to determine whether glufosinate ammonium has a common mechanism of toxicity with other substances. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity (i.e. organophosphates), EPA has not made a common mechanism of toxicity finding as to glufosinate ammonium and any other substances and glufosinate ammonium does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that glufosinate ammonium has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

***Sierra Club Comment E - Safety Determination U.S. population.*** We believe that EPA has not done an adequate scientific job with respect to its safety determination for the U.S. population. By using what EPA claims (and may be a flawed set of assumptions) are the conservative assumptions described above and based on the completeness and reliability of the toxicity data, it is concluded that chronic dietary exposure to the registered and proposed uses of glufosinate-ammonium will utilize at most 25% of the chronic RfD for the U.S. population. We disagree with EPA's assumption that the actual exposure is likely to be significantly less than predicted by this analysis as data and models that are more realistic are developed. We disagree with EPA's assumption that exposures below 100% of the reference dose (RfD) are generally assumed to be of no concern because the RfD represents the level at or below which daily aggregate exposure over a lifetime will not pose appreciable risk to human health. We dispute that the acute population of concern, female 13+ utilizes 34% of the acute RfD. We disagree with EPA's-assumption that this is a Tier One highly conservative assessment and actual exposure is likely to be far less. Drinking water levels of comparison based on dietary exposures are greater than highly conservative estimated levels, and would be expected to be well below the 100% level of the RfD, if they occur at all, assuming that EPA's set of assumptions are reasonably accurate which they may not be. We believe that EPA has erroneously concluded that it is not appropriate to aggregate non-dietary exposures with dietary exposures in a risk assessment because the toxicity end-points are different. We strongly dispute EPA's concluding assumption that there is a

reasonable certainty that no harm will occur to the U.S. population from aggregate exposure (food, drinking water and nonresidential) to residues of glufosinate-ammonium and metabolites.

*HED Response:* Contrary to what was written in the NOF, HED did aggregate dietary (food + drinking water) and residential exposures. Glufosinate ammonium is currently registered for application in the residential setting for lawn renovation and spot treatment purposes. Since the lawn renovation use resulted in exposures greater than HED's level of concern, revocation of this use was recommended. Therefore, aggregate exposures were conducted by combining dietary exposure and residential exposure resulting from the spot treatment use. The resulting combined exposures were subtracted from the appropriate dose and drinking water levels of comparison (DWLOCs) were calculated and compared to estimated environmental concentrations (EECs) in groundwater and surface water. The EECs were generated using SCIGROW (groundwater) and PRZM-EXAMS (surface water). SCIGROW is a regression model designed to estimate a screening level of a pesticide concentration at an agricultural site which is highly vulnerable to leaching due to permeable soil overlaying shallow ground water. PRZM-EXAMS is used to estimate concentration that might occur in vulnerable surface water (assumes 87% of the basin is cropped and entire cropped area is treated). Both models assumed 3 applications at 1.5 lbs ai/acre (highest registered/proposed rate). The resulting EECs were less than the DWLOCs indicating aggregate exposures are less than HED's level of concern.

HED concluded that provided the petitioner revokes the lawn renovation use, a conditional registration was acceptable. Unconditional registration may be established upon submission of data addressing the toxicology data gaps (see D290086, T. Bloem *et al*, 7-Aug-2003).

***Sierra Club Comment G - Additional issues not apparently being addressed by EPA such as negative impacts on beneficial insects.*** Bystander or beneficial insects have been detrimentally effected by the herbicide. Kutlesa and Caveny (6) found that the herbicide had a number of neurotoxic impacts on the skipper butterfly at levels of herbicide experienced in the field. Ahn et al (7) found that glufosinate was toxic to some but not all predatory insects at levels of the herbicide experienced in the field. Studies showing that helpful predatory insects or bystander insects are poisoned by. the herbicide seem to have been ignored by regulators of the herbicide.

*HED Response:* This comment will be addressed separately.

***Sierra Club Comment H - Additional issues not apparently being addressed by EPA such as glufosinate residues in other crop varieties.*** Muller et al (8) studied glufosinate metabolites in transgenic and unmodified sugar beet, carrot, purple foxglove and thorn apple, and they found that unmodified (i.e. non-genetically engineered) crops contained glufosinate mainly while GM crops contained higher levels of glufosinate and acetyl glufosinate. Beriault et al (9) studied phloem transport of glufosinate and acetylglufosinate in canola in GM canola and unmodified canola and found that both chemicals were highly mobile.

*HED Response:* Common toxicity studies conducted with glufosinate ammonium, N-acetyl-glufosinate, and 3-MP indicate that N-acetyl-glufosinate and 3-MP exhibit toxic effects at doses equal to or greater than glufosinate ammonium. Based on these toxicity studies, HED concluded that N-acetyl-glufosinate, and 3-MP are not likely to be more toxic than glufosinate ammonium (risk assessment assumes they are of equal toxicity to parent; see D282757, 9-May-2002

memorandum). The field trial data were submitted for the transgenic crops monitored for residues of glufosinate ammonium, N-acetyl-glufosinate, and 3-MP in/on all food/feed commodities. Therefore, the higher residues in transgenic crops and/or greater mobility of the residues of concern has been taken into consideration.

attachments:
HIARC document - TXR No. 0051833, 17-April-2003
MARC decision document - D282757, 9-May-2002
Risk Assessment - D290086, 7-Aug-2003

cc without attachments: T. Bloem (RAB1)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**TXR NO. 0051833**

**DATE:** **April 17, 2003**

**MEMORANDUM**

**SUBJECT:** **GLUFOSINATE AMMONIUM- 3rd** Report of the Hazard Identification Assessment Review Committee.

**FROM:** Brenda Tarplee, Senior Scientist
Science and Information Management Branch
Health Effects Division (7509C)

**THROUGH:** Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

**TO:** Thomas Bloem, Risk Assessor
Health Effects Division (7509C)

PC Code: 128850

On March 27, 2003, in response to questions raised by the Office of General Council, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) was asked to reevaluate the need for and size of the Database Uncertainty Factor (UFDB) applied in the Glufosinate ammonium risk assessment for the lack of: the developmental neurotoxicity (DNT) study; comparative glutamine synthetase (GS) measures; and repeat ACN with GS measures. The conclusions of the committee are presented in this report.

## Committee Members Participating

William Burnam, Elizabeth Doyle, Bill Dykstra, Pamela Hurley, Elizabeth Mendez, Ayaad Assaad, John Liccione, Jonathan Chen, P. V. Shah, Jess Rowland, and Brenda Tarplee.

Meeting materials prepared by: Brenda Tarplee, Senior Scientist, SIMB

## INTRODUCTION

On June 4, 2002, and June 11, 2002 , the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Glufosinate ammonium with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996 according to the 2002 OPP 10X guidance document (TXR NO. 0050900).

On March 27, 2003, in response to questions raised by the Office of General Council, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) was asked to reevaluate the need for and size of the Database Uncertainty Factor (UFDB) applied in the Glufosinate ammonium risk assessment for the lack of: the developmental neurotoxicity (DNT) study; comparative glutamine synthetase (GS) measures; and repeat ACN with GS measures. The committee conclusions made during all of the above meetings are presented in this report.

## FQPA HAZARD CONSIDERATIONS

### 1. Adequacy of the Toxicity Data Base

The following studies are available for FQPA assessment:

Acute Neurotoxicity Study in White Longhorn hen
Acute Neurotoxicity Study in Rats (acceptable/non-guideline)
Two Subchronic Neurotoxicity Studies in Rats (new study -acceptable/non-guideline)
Developmental Toxicity Study in Rats (acceptable/guideline)
Developmental Toxicity Study in Rabbits (acceptable/guideline)
2- Generation Reproduction Study in Rats (acceptable/guideline)

In addition, following studies are available on Glufosinate ammonium metabolites:

Acute Oral Neurotoxicity- N-Acetyl-L-Glufosinate disodium (acceptable/non-guideline)
Subchronic Neurotoxicity Study- N-Acetyl-L-Glufosinate disodium (acceptable/non-guideline)
Developmental Toxicity Study in Rats- HOE 099730 (acceptable/guideline)
Developmental Toxicity Study in Rats- HOE 061517 (acceptable/guideline)
Developmental Toxicity Study in Rabbits- HOE 058192 (L- Isomer) (acceptable/guideline)
Developmental Toxicity Study in Rabbits- HOE 099730 (acceptable/guideline)
Developmental Toxicity Study in Rabbits- HOE 061517 (acceptable/guideline)

The toxicology database for Glufosinate ammonium is not considered to be complete. On June 11, 2002, the HIARC identified the following data gaps: acute neurotoxicity study conducted in the rat which includes glutamine synthetase (GS) activity measurement in the liver, kidneys, and

brain; and comparative glutamine synthetase activity measurement in the liver, kidneys, and brain of the pups and mothers in the developmental neurotoxicity (DNT) study conducted in the rat (the DNT was previously requested in 1999). The HIARC also requested additional data to confirm that liver and kidney changes - observed in the absence of histopathological changes - are an adaptive response and not an adverse effect. Kidney and liver function assays should be performed in addition to glutamine synthetase activity measurements. The HIARC concluded that a new subchronic neurotoxicity study in rats **is not** required since it is not expected to provide additional information for regulatory purposes (the doses selected for risk assessment are lower than those that will be tested in a new study)(TXR NO. 0050900).

**2. Evidence of Neurotoxicity**

The HIARC concluded that there is a concern for neurotoxicity resulting from exposure to Glufosinate ammonium.

### 2.1. Acute Oral Neurotoxicity- Glufosinate Ammonium

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45190704), groups of 10 male and 10 female Wistar rats were administered glufosinate ammonium (50.2%; Batch No. C01284032) as a single oral gavage dose of 0, 10, 100, or 500 mg a.i./kg. Doses were based on range-finding studies which were summarized in MRID 45179102. Animals were observed for 14 days post-dosing and body weights were determined weekly. Learning and memory were assessed in a water maze on all animals pre-test and on days 1, 7 and 14 after dosing. All animals were necropsied and fixed by *in situ* perfusion. Neuropathological examinations were conducted on brain, spinal cord, sciatic nerve, and tibial nerve. Positive control data for the water maze test and for neuropathology were submitted in MRID 45297001.

All animals survived to scheduled termination. No clinical signs of toxicity were observed in any animal during the study. No treatment-related differences in absolute body weights, body weight gains, or food consumption were observed between the treated and control groups during the study. No treatment-related lesions were found at necropsy or with microscopic examination of the nervous tissues.

Learning and memory were not affected in either sex by treatment with the test article. The percentage of animals with correct responses was similar between the treated and control groups at all testing days.

**Under the conditions of this study, the NOAEL for male and female rats is ≥500 mg/kg and the LOAEL was not identified.**

This acute oral neurotoxicity study is classified as **Acceptable/Nonguideline** and does not satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats. When considered in conjunction with MRID 45190703, which used the same doses and evaluated rats on a functional observational battery and automated motor activity test, although

together they contain all of the essential elements of a guideline acute neurotoxicity study, both are considered inadequate for the same reason, i.e., the lack of an LOAEL or a limit dose.

The major deficiency was that an LOAEL (or a limit dose) was not established.

## 2.2. Acute Oral Neurotoxicity- Glufosinate Ammonium

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45190703), groups of 10 male and 10 female Wistar rats were administered glufosinate ammonium (50.2%; Batch No. C01284032) as a single oral gavage dose of 0, 10, 100, or 500 mg a.i./kg. Doses were based on range-finding studies which were summarized in MRID 45179102. Animals were observed for 14 days post-dosing and body weights were determined weekly. Functional observational battery (FOB) and motor activity tests were conducted on all animals pre-test and on days 1, 7 and 14 after dosing. All surviving animals were necropsied. Neuropathological examinations were not performed.

All animals survived to scheduled termination. No treatment-related clinical signs of toxicity or differences in absolute body weights, body weight gains, or food consumption were observed between the treated and control groups during the study.

No differences in the endpoints observed during the FOB were found between the treated and control groups. Fore- and hind-limb grip strengths, landing foot splay, body temperature, and rearing of the males and females were similar between the treated and control groups throughout the study. No treatment-related differences were observed in locomotor activity between the treated and control groups of either sex. No abnormalities were observed at gross necropsy.

A positive control study with TOTP (MRID 45297002) failed to provide evidence of the sensitivity of the test procedures.

**Under the conditions of this study, the NOAEL for male and female rats is ≥500 mg/kg and a LOAEL was not identified.**

This acute oral neurotoxicity study is classified as **Acceptable/Nonguideline** and does not satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats. In this study, a LOAEL was not established at the highest dose tested (500 mg/kg), which is below the limit dose of 2000 mg/kg.

A companion acute neurotoxicity study (MRID 45190704) assessed Wistar rats (10 rats/sex /dose) treated with same doses on a water maze learning and memory test and evaluated 10 rats/sex/dose for neuropathology after *in situ* perfusion. This second acute oral neurotoxicity study is also classified as **Acceptable/Nonguideline** in which the LOAEL was not established at the highest dose tested (500 mg/kg; below the limit dose of 2000 mg/kg).

Together, for the reasons noted, these 2 studies, while they contain all of the essential elements of a guideline neurotoxicity study and a learning and memory test, do not satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats.

### 2.3. Acute Oral Neurotoxicity- N-Acetyl-L-Glufosinate Disodium (metabolite)

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45190702), groups of 10 male and 10 female Wistar rats were administered N-acetyl-L-glufosinate disodium (33.8%; Batch No. Lot2+3/Fass1-4/17714) as a single oral gavage dose of 0, 100, 1000, or 2000 mg a.i./kg. Doses were based on a range-finding study which was summarized in MRID 45179102. Animals were observed for 14 days post-dosing and body weights were determined weekly. Learning and memory were assessed in a water maze on all animals pre-test and on days 1, 7 and 14 after dosing. The rationale for the time to peak effect was inadequate. All surviving animals were necropsied and fixed by *in situ* perfusion. Neuropathological examinations were conducted on brain, spinal cord, sciatic nerve, and tibial nerve. Positive control data for the water maze test and for CNS pathology were submitted in MRID 45297001.

All animals survived to scheduled termination. In the high-dose groups, sedation was observed in 10/10 males and 4/10 females and ruffled fur was evident in 7/10 males and in 5/10 females. These clinical signs were noted beginning at 2 hours after treatment in males and 4 hours after treatment in females with most continuing to the end of the 10-hour observation period of test day 1. In addition, 10/10 high-dose males and 9/10 high-dose females had diarrhea on the day of treatment. Clinical signs of toxicity were resolved one day after treatment. No clinical signs were observed in the low- or mid-dose groups at any time during the study.

No treatment-related differences in absolute body weights, body weight gains, or food consumption were observed between the treated and control groups during the study. Performance in the water maze was not affected in either sex by NAG exposure. The percentage of animals with correct responses was similar between the treated and control groups at all testing days.

No treatment-related lesions were found at necropsy or with microscopic examination of the nervous tissues.

**Under the conditions of this study, the NOAEL for male and female rats is 1000 mg/kg. The LOAEL is 2000 mg/kg based on clinical signs of toxicity including sedation, ruffled fur, and diarrhea.**

This acute oral neurotoxicity study is classified as **Acceptable/Nonguideline** and does not satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats. When considered together, MRID 45190701 and 45190702 contain all of the essential elements of a guideline neurotoxicity study and a learning and memory test. Submission of acceptable positive control data for neuropathology and maze learning from the testing laboratory could lead to upgrade of the study, when combined with MRID 45190701.

## 2.4. Acute Oral Neurotoxicity- N-Acetyl-L-Glufosinate Disodium (metabolite)

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45190701), groups of 10 male and 10 female Wistar rats were administered N-acetyl-L-glufosinate disodium (33.8%; Batch No. Lot2+3/Fass1-4/17714) as a single oral gavage dose of 0, 100, 1000, or 2000 mg a.i./kg. Doses were based on a range-finding study which was summarized in MRID 45179102. Animals were observed for 14 days post-dosing and body weights were determined weekly. Functional observational battery (FOB) and motor activity tests were conducted on all animals pre-test and on days 1, 7 and 14 after dosing; a more precise time to peak effect was not determined. All surviving animals were necropsied.

All animals survived to scheduled termination. In 10/10 high-dose males and females, sedation and ruffled fur were noted beginning at 3 hours after treatment in males and 4 hours after treatment in females and continuing to the end of the 10-hour observation period of test day 1. In addition, 9/10 high-dose males and 8/10 high-dose females had diarrhea on the day of treatment. Clinical signs of toxicity were resolved one day after treatment. No clinical signs were observed in the low- or mid-dose groups at any time during the study. No consistent treatment-related differences in absolute body weights, or food consumption were observed between the treated and control groups during the study, but there were significant decreases in body weight gain in male rats between pre-dosing and day 8 at the mid-dose (20%) and the high dose (27%). No differences in the endpoints observed during the FOB, one day after dosing, were found between the treated and control groups. Fore- and hind-limb grip strengths, landing foot splay, body temperature, and rearing of the males and females were similar between the treated and control groups throughout the study. No dose- or treatment-related differences were observed in locomotor activity between the treated and control groups of either sex. A positive control study with TOTP (MRID 45297002) failed to provide evidence of the sensitivity of the test procedures.

**The LOAEL is 1000 mg/kg based on decreased body weight gain (20%) in male rats.**

**The NOAEL is 100 mg/kg.**

This acute oral neurotoxicity study is classified as **Acceptable/Nonguideline** and does not satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200(§81-8)] in rats. When considered together, MRID 45190701 and 45190702 contain all of the essential elements of a guideline neurotoxicity study and a learning and memory test. Submission of acceptable positive control data for observations and motor activity could lead to upgrade of the study, when combined with MRID 45190702.

## 2.5. Repeat Dose Oral Neurotoxicity- Glufosinate ammonium and N-Acetyl-L-Glufosinate Disodium (metabolite)

EXECUTIVE SUMMARY: In a 37-day repeat-dose dietary neurotoxicity study (MRID 45179101), glufosinate-ammonium (GA) (Batch No.: C01284032, 50.2% w/w) was administered to 15 HanIbm:Wistar rats/sex/dose at dietary levels of 0, 20, 200, or 2000 ppm. Nominal doses in terms of active ingredient were 0.0, 1.5, 14.9, and 143.3 mg/kg/day for males and 0.0, 1.8, 17.1, and 161.5 mg/kg/day for females. Additional groups of 15 rats/sex were administered N-

acetyl-L-glufosinate disodium (NAG) (Lot2+3/Fass1-4/17714), a plant metabolite of GA, for 38 days at dietary levels of 0, 20, 200, or 2000 ppm. Nominal doses in terms of active ingredient were 1.6, 15.5, and 158.9 mg/kg/day for males and 1.75, 17.7, and 179.4 mg/kg/day for females. Dietary levels were chosen on the basis of a range-finding study (MRID 45179102). Functional observational battery (FOB), motor activity, and a water maze test were performed pretreatment and during weeks 2 and 4 on 10 rats/sex/group. At the completion of the study, 10 rats/sex/group in the control and high-dose groups were sacrificed, perfused, and brain and nervous system tissues were examined microscopically. The remaining 5 rats/sex/group were necropsied, and the livers, kidneys, and brains weighed and analyzed for glutamine synthetase activity.

No deaths occurred during treatment with either chemical and there were no clinical signs attributable to treatment. There were no effects on body weight or food consumption. For the FOB, water maze, and motor activity, there were no differences between the control group and any group administered either GA or NAG. There were no gross or histopathological findings that could be attributed to treatment. In rats administered GA, glutamine synthetase activity was statistically significantly reduced in a concentration-related manner in the livers of male and female rats in all dose groups and in a non-concentration related manner in the kidney of male rats in all dose groups. Inhibition of glutamine synthetase activity was also observed in the brain of male rats in the 200 and 2000 ppm treatment groups (93 and 75% of the control value, respectively), and in female rats in the 2000 ppm group (73% of the control value). Inhibition of glutamine synthetase activity was much less pronounced in rats administered NAG, with statistically significant reductions in the liver of males in the 200 and 2000 ppm groups, in the liver of females in the 2000 ppm dose group, and in the kidney of males in the 2000 ppm dose group. On June 4, 2002 the HIARC considered that the inhibition of glutamine synthetase in the livers and kidneys of rats are adaptive changes and not an adverse effect.

**The LOAEL for glufosinate-ammonium was 200 ppm (14.9-17.1 mg/kg) based on glutamate synthetase inhibition (7%) in the brains of males.**

**The NOAEL for glufosinate-ammonium was 20 ppm in the diet ( 1.5 mg/kg/day in male rats and 1.8 mg/kg/day in female rats) based on the inhibition of glutamate synthetase in the brain.**

**The NOAEL for N-acetyl-L-glufosinate disodium was >2000 ppm in the diet (158.9-179.4 mg/kg/day). The LOAEL for N-acetyl-L-glufosinate disodium was not established.**

This study is considered to be **Acceptable/Nonguideline** as a 90-day neurotoxicity feeding study and does not fulfill FIFRA guideline requirements for a subchronic (90-day) neurotoxicity study in rats **(OPPTS 870.6200b, §82-7)**. Dose selection for both GA and NAG, and the study duration of 37-38 days were judged to be inadequate.

### 2.6. Repeat Dose Oral Neurotoxicity- Glufosinate ammonium

EXECUTIVE SUMMARY: In a 90-day feeding neurotoxicity study (MRID 42768201), Hannover-derived Wistar rats (10/sex/dose) received glufosinate ammonium at dietary

concentrations of 0, 7500, 10000 or 20000 ppm (control, LDT, MDT, HDT) for 13 weeks. The values of compound intake for treated animals were calculated to be 521.45, 685.95 and 1351.09 mg/kg/day for males and 573.79, 740.57 and 1442.64 mg/kg/day for females.

Two female HDT rats died. Also at HDT, the test compound produced clinical signs such as sedation, lateral recumbency, hunched posture, dyspnea, ruffed fur and emaciation in males and females. No deaths or clinical signs were seen at MDT or LDT. At LDT, increases in the incidence of decreased exploratory activity, decreased alertness, decreased startle response and meiosis were seen during the first four weeks of treatment. MDT rats also showed similar signs in addition to increased body tone, increased fearfulness and increased pain response. HDT rats showed similar effects to MDT rats, but the responses were more severe and persistent. In addition, diarrhea, increased vocalization, and apathy were also found in HDT rats. One HDT female showed signs of rearing, convulsive twitches and profuse salivation. **The LOAEL for this study is 7500 ppm (521.45/573.79 mg/kg/day in M/F) based on increases in the incidence of decreased exploratory activity, decreased alertness, decreased startle response and meiosis. The NOAEL has not been established.**

This study was classified as **supplementary** and does not satisfy the requirements for a subchronic neurotoxicity study (82-7), because the neural histopathological data were not available making it impossible to correlate findings of the functional observation battery with histopathology; and because the study did not provide measurement of grip strength, hind limb splay or automated motor activity measurements. Study is **upgradable** upon satisfactory submission of the missing required data.

**3. Developmental Toxicity Study Conclusions**

3.1 Prenatal Developmental Toxicity Study-Rabbits

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 40345611, 41144703), groups of 15 pregnant female Himalayan rabbits were administered by gavage HOE 039866 at doses of 0., 2.0, 6.3 or 20.0 mg/kg/day from days seven to 19 of pregnancy.

There was a decrease in body weight (6 - 8%, $p \leq 0.05$), body weight gain (37%, $p \leq 0.05$) and food consumption (39%, $p \leq 0.05$) in 20 mg/kg dams. A drop in food consumption (15%, $p \leq 0.05$) was also seen at 6.3 mg/kg. In the 20 mg/kg group, there were increased kidney weights (11%, $p \leq 0.05$) in the dams. Also at 20 mg/kg/day there was an increase in the number of dead fetuses/litter (0.55/litter vs. 0.00/litter in controls, reported as outside the normal range") and a 4% decrease in mean fetal body weight, also reported as "outside the normal range". Increased incidence of incomplete or absent ossification of skeletal bones in fetuses were observed in the 6.3 and 20.0 mg/kg groups (3 fetuses in 2 litters at 6.3 mg/kg, 9 fetuses in 4 litters at 20 mg/kg. Statistical analysis was not reported). On June 4, 2002, the HIARC considered that the statistically significant increased kidney weights seen at 20 mg/kg/day (HDT) as an adaptive response and not an adverse effect.

Based on the findings presented in this report, the **NOAEL for maternal toxicity was 6.3 mg/kg/day. The LOAEL is 20.0 mg/kg/day based on reduced food consumption, body weight and weight gains. The developmental NOAEL was 6.3 mg/kg/day based on decreased body weights and fetal death at 20 mg/kg/day.**

This study is **classified as Acceptable (Guideline)** and meets the requirements for a developmental toxicity study (83-3b) in the rabbit.

## METABOLITES

### 3.2 Prenatal Developmental Toxicity Study-Rabbits

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076205) HOE 099730 (92.4% a.i.) was administered to 15 Himalayan rabbits/dose in distilled water by gavage at dose levels of 0, 64, 160, or 400 mg/kg/day from days 6 through 18 of gestation.

Minimal maternal toxicity was demonstrated by reduced feed consumption (↓22-24%; $p<0.05$) in the 160 mg/kg/day group and in the 400 mg/kg/day group (↓30-40%; $p<0.05$) during treatment days 6-13 and 13-19. There were no treatment-related effects in mortality, clinical signs, body weight, or cesarean section parameters. **The maternal LOAEL was 160 mg/kg/day based on reduced feed consumption. The maternal NOAEL was 64 mg/kg/day.**

A uni- or bilateral extra rib at the 13th thoracic vertebra was observed in the 160 mg/kg/day group; **based on this finding the developmental LOAEL was 160 mg/kg. The developmental NOAEL was 64 mg/kg/day.**

Usually, data are required to confirm the nominal concentrations of the administered doses. Without these data, the study would have been classified as unacceptable. However, the test substance is a metabolite of glufosinate ammonium which has an adequate developmental toxicity data base. In addition, this study was submitted for verification of the NOAEL and LOAEL provided by the registrant to show that toxicity of various metabolites is less than that of the parent compound. Under the circumstance, this study is considered as **acceptable/Nonguideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in rabbits.

### 3.3 Prenatal Developmental Toxicity Study-Rabbits

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076210) Hoe 061517 (a metabolite of glufosinate ammonium) (99.6% a.i.) in distilled water was administered to 15 Hoe:HIMK (SPFWiga) Himalayan rabbits/dose/group by gavage at dose levels of 0, 50, 100, or 200 mg/kg/day from days 6 through 18 of gestation.

Maternal toxicity was demonstrated at 100 mg/kg/day, as a dose-related increase in abortions (7% vs. 0% in controls) and mortality (7% vs. 0% in controls), clinical signs (disequilibrium), and reductions in food and water consumption, body weight gain, and fecal output. At 200 mg/kg/day, maternal toxicity was demonstrated by treatment-related clinical signs of toxicity (disequilibrium, and/or straddled fore-limbs), increases in abortions (27% vs. 0% in controls) and

mortality (33% vs. 0% in controls), reductions in body weight gain, food and water consumption, and fecal output. In addition, treatment-related gross pathology was noted in the kidneys of the high-dose animals and was characterized as uneven, rough surface of one high-dose dam, and light-brown coloring of the renal cortex of three of the four aborting high-dose dams. Corroborative treatment-related increases in the mean kidney weights was also noted at 200 mg/kg/day.

At 50 mg/kg level, no treatment-related deaths or effects were reported. **The maternal LOAEL is 100 mg/kg/day, based on increased abortions and mortality and reductions in food and water consumption, body weight gain, and fecal output. The maternal NOAEL is 50 mg/kg/day.**

There were no treatment-related effects noted in developmental parameters at any dose level. **A developmental LOAEL was not observed (>200 mg/kg/day). The developmental NOAEL is 200 mg/kg/day.**

This developmental toxicity study in the rabbit is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in the rabbit. In order to upgrade the study, the sponsor must submit data confirming the nominal concentrations of the administered doses and the stability of the test substance in distilled water.

**ISOMER**

**3.4 Prenatal Developmental Toxicity Study-Rabbits**

EXECUTIVE SUMMARY: In a rabbit developmental toxicity study (MRID 43829405), groups of mated Chinchilla rabbits (16/dose group) received Hoe 058192 (88.2% a.i.) by gavage at dose levels of 1.25, 2.50, and 5.00 mg/kg/day from gestation days 6 to 18 inclusive.

In the high dose group (5.00 mg/kg), one treatment-related death occurred, and prior to death this dam showed clinical signs of severe spasms, lateral recumbency, and muscle twitching. In addition two other high dose dams also exhibited signs of abortion; these two dams were sacrificed prior to termination of the study. A dose-related decrease in body weight gain and food consumption was seen in the mid and high dose dams. The absolute kidney weights in the high dose dams were increased. **Based on the decrease in body weight gains and food consumption, neurotoxic signs, and abortions, the LOAEL and NOAEL for maternal toxicity were 2.5 and 1.25 mg/kg, respectively**.

A statistically significant increase in post-implantation loss/fetal resorptions was found in mid and high dose groups. No increases in the incidence of external, visceral or skeletal malformations or skeletal variations (altered growth) were found. **The LOAEL for developmental toxicity is 2.5 mg/kg based on an increase in post-implantation loss (fetal resorptions); NOAEL, 1.25 mg/kg**.

This study is classified as **acceptable** and satisfies the guideline requirements for a developmental toxicity study in rabbits (§ 83-3b).

## 3.5 Prenatal Development Toxicity Study- Rats

EXECUTIVE SUMMARY: In a teratology/post-natal study (MRID 00142445, 00142445 [Accession No.072965]) groups of 20 pregnant female Wistar rats were administered by gavage HOE 039866 (glufosinate ammonium, 96.9 a.i.)at doses of 10, 50 and 250 mg/kg/day from days seven to 16 of pregnancy. The dams were allowed to deliver normally, and after birth the offspring were observed for 21 to 23 days. In a second teratology/post-natal study (MRID 00151499, 00151500 [Accession No. 073916]) groups of 20 pregnant female Wistar rats were administered by gavage HOE 039866 at doses of 0.50, 2.24 and 10.00 mg/kg/day from days seven to 16 of pregnancy. The dams were allowed to deliver normally, and after birth the offspring were observed for 21 to 23 days. This study was conducted since the first study showed maternal and developmental effects at all dose levels and did not give a NOAEL. In a third teratology/post-natal study (MRID 40345610) groups of 20 pregnant female Wistar rats were administered by gavage HOE 039866 at doses of 0.50, 2.24 and 10.00 mg/kg/day from days seven to 16 of pregnancy. The dams were allowed to deliver normally, and after birth the offspring were observed for 21 to 23 days. This third study was done to confirm the results of the second study (which it successfully did), because the second study showed results that contradicted the first study, i.e., the second study showed no maternal or developmental effects at 10 mg/kg/day, whereas the first study showed maternal and developmental effects at this level.

The combined results of the three studies (along with comparison with historical control data) are as follows: There were observed no significant differences in clinical observations, food consumption, body weights, implantations, resorption, and autopsy findings between treated and control dams. In offspring, survival rates and body weight (at birth or sacrifice) were comparable between those from treated groups and those from controls. No increases in the incidence of soft tissue and skeletal anomalies were found in the offspring of the treated animals relative to those of the controls. Dilated renal pelvis and/or hydroureter was observed in fetuses at 250 mg/kg/day; in dams at 50 mg/kg/day, increased vaginal bleeding and hyperactivity were observed. Based on the findings presented in these reports, **the no observed effect level (NOAEL) for maternal toxicity is 10 mg/kg/day; the LOAEL is 50 mg/kg/day based on vaginal bleeding and hyperactivity in dams. In the fetus, the NOAEL is 50 mg/kg/day, based on dilated renal pelvis at the LOAEL of 250 mg/kg/day**. These conclusions have been confirmed by the HED Peer Review Committee (TXR 013309, 013310).

These data are classified as **Acceptable (Guideline)** and meets the requirements for a developmental toxicity study (83-3a) in the rat.

## METABOLITES

3.6 Prenatal Development Toxicity Study- Rats

EXECUTIVE SUMMARY: In a developmental toxicity study (limit test) (MRID 44076204) Hoe 099730 00 ZC75 0001 (74.7% a.i.) a glufosinate ammonium metabolite, was administered once daily via oral gavage to 20-21 female Wister rats at dose levels of 0 or 1000 mg/kg/day from days 7 through 16 of gestation.

No maternal toxicity was observed at the 1000 mg/kg/day dose. There were no treatment-related effects in mortality, body weight, feed consumption, gross pathology, or cesarean section parameters. **The maternal LOAEL was not observed. The maternal NOEL is >1000 mg/kg/day.**

There was no evidence of treatment-related developmental toxicity in the treatment group. There were no treatment-related effects found upon cesarean section examinations. Numbers of corpora lutea, implantations, live fetuses, resorptions, and fetal weights were similar among treated and untreated animals. There were no treatment-related effects found upon external, visceral, and skeletal examination of the fetuses. **The developmental LOAEL was not observed. The developmental NOEL is >1000 mg/kg/day.**

Dosing was considered adequate because the dose of 1000 mg/kg/day represents a "limit dose".

The developmental toxicity study in the rat is classified **acceptable/guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 (a) in rats.

**3.7 Prenatal Development Toxicity Study- Rats**

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076209) Hoe 061517 (a metabolite of glufosinate ammonium; 99.6% a.i.) in distilled water was administered by gavage to 20 presumed pregnant Wistar rats/dose at dose levels of 0, 100, 300, or 900 mg/kg/day from days 6 through 17 of gestation

Maternal toxicity was demonstrated at 900 mg/kg/day, maternal toxicity was demonstrated by one death, treatment-related clinical findings (persistent piloerection and/or increased urinary output), increased absolute kidney weights. **The maternal LOAEL is 900 mg/kg/day; NOAEL, 300 mg/kg/day.**

At 900 mg/kg/day, increases in the incidence of total litter loss and in the fetal and litter incidence of wavy and/or thickened ribs were found. There were additional treatment-related effects noted in developmental parameters. **Therefore, the developmental LOAEL for developmental toxicity is 900 mg/kg/day; NOAEL, 300 mg/kg/day.**

The developmental toxicity study in the rat is classified as **Acceptable/non-guideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat. It should be noted that this

study was submitted for verification of the NOEL and LOAEL provided by the registrant to show that the toxicity of various metabolites is less than that of the parent compound.

**4. Reproductive Toxicity Study Conclusions**

EXECUTIVE SUMMARY: In a 2 generation reproduction study (MRID 40345612), Glufosinate ammonium (HOE 039866 Technical, 95.3% a.i.) was administered to groups of 30 male and 30 female Wistar/Han rats in the diet at concentrations of 0, 40,, 120 or 360 ppm (approximately 2.0, 6.0, 18.0 mg/kg) for two generations. Two litters were produced in the first generation ($F_{1a}$ and $F_{1b}$) and two litters in the second generation ($F_{2a}$ and $F_{2b}$) Animals were given test or control diet for at least 14 weeks then mated within the same dose group. $F_1$ animals were chosen from the $F_{1b}$ litters and weaned on the same diet as their parents. At least 26 litters/group were produced in each generation. All animals were exposed to test material either in the diet or during lactation until sacrifice.

At 120 and 360 ppm, significant ($p \leq 0.05$) increases in kidney weights were seen in both sexes and both generations. No effects were seen on mating or pregnancy indices. At 360 ppm there was a significant ($p \leq 0.05$) reduction in the mean number of viable pups/litter in all generations (-21% to -37%) with the exception of the $F_{2a}$ generation where the reduction (-11%) was not statistically significant. Surviving pups and the weight of survivors were unaffected by treatment. No other reproductive or developmental effects were seen at the low-and mid-dose level. There were no dose- or treatment-related clinical signs of toxicity in the offspring of either generation. On June 4, 2002, the HIARC considered that the increased kidney weights seen in both sexes and generations as an adaptive response and not an adverse effect.

**The LOAEL for systemic toxicity was not established. The systemic toxicity NOAEL is ≥ 360 ppm (18.0 mg/kg/day, HDT).**

**The LOAEL for reproductive/developmental toxicity is 360 ppm (18 mg/kg/day) based on decreased number of viable pups in all generations. The NOAEL is 120 ppm (6.0 mg/kg/day).**

This study is classified as Acceptable and satisfies the guideline requirement for a reproduction study (83-4) in rats.

**5. Additional Information from Literature Sources** No relevant citations were found.

**6. Pre-and/or Postnatal Toxicity**

The HIARC concluded that the available toxicity data for Glufosinate ammonium indicate that the metabolites elicit similar types of effects but at higher doses than the parent compound (i.e., are considered to be less toxic). The single developmental toxicity study conducted with the L-isomer also demonstrates similar effects but at lower doses than Glufosinate ammonium. However, the L-isomer is not the registered active ingredient. Therefore, the FQPA assessment performed by the HIARC is based on the results of studies conducted with the Glufosinate

ammonium. The HIARC concluded that there is a concern for pre- and/or postnatal toxicity (i.e., susceptibility was demonstrated) following exposure to Glufosinate ammonium.

A. Determination of Susceptibility

There is no qualitative or quantitative evidence of increased susceptibility in the developmental toxicity study conducted in rats. Qualitative evidence of increased susceptibility is demonstrated in the rabbit developmental toxicity study since fetal deaths were observed in the presence of lesser maternal toxicity at the same dose. There is also quantitative evidence of increased susceptibility in the rat 2-generation reproduction study. In this study, a decrease in the number of viable pups was observed in the absence of parental toxicity at any dose.

B. Degree of Concern Analysis and Residual Uncertainties

Since there is qualitative evidence of increased susceptibility of the young following exposure to Glufosinate ammonium, HIARC performed a Degree of Concern Analysis to: 1) determine the level of concern for the effects observed when considered in the context of all available toxicity data; and 2) identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of this chemical. If residual uncertainties are identified, HIARC examines whether these residual uncertainties can be addressed by a special FQPA safety factor and, if so, the size of the factor needed. The results of the HIARC Degree of Concern analysis for Glufosinate ammonium follow.

In the rabbit developmental toxicity study, qualitative susceptibility was evidenced at the highest dose tested as a decrease in mean fetal body weight and an increase in the number of dead fetuses/litter in the presence of maternal toxicity (decreased body weight, body weight gain, and food consumption). Considering the overall toxicity profile and the doses and endpoints selected for risk assessment for Glufosinate ammonium, the HIARC characterized the degree of concern for the effects observed in this study as low, noting that the fetal effects of concern occurred only at the highest dose tested and that a clear NOAEL for the effects was established. No residual uncertainties were identified. The NOAEL of 6.3 mg/kg/day identified in this study is used to establish the acute Reference Dose (aRfD) for the Females 13-50 population subgroup.

In the 2-generation reproduction study, quantitative susceptibility was evidenced as reduction in the mean number of viable pups/litter in all generations (with the exception of the $F_{2a}$ generation where the reduction was not statistically significant) in the absence of parental toxicity at any dose level (the HIARC considered the significant increases in kidney weights seen at the mid and high dose in both sexes and both generations to be an adaptive response and not an adverse effect). Considering the overall toxicity profile and the doses and endpoints selected for risk assessment for Glufosinate ammonium, the HIARC characterized the degree of concern for the effects observed in this study as low, noting that clear NOAELs and LOAELs are identified for the offspring effects of concern

and the dose-response well-characterized. No residual uncertainties were identified. The extrapolated NOAEL of 2.0 mg/kg/day used to establish the chronic Reference Dose (cRfD) for all populations is protective of the effects seen in the young in the reproduction study (offspring LOAEL of 18 mg/kg/day is nearly 10-fold higher).

C. Hazard-based Special FQPA Safety Factor(s):

The HIARC recommended that the special FQPA Safety Factor can be removed (**1x** ) because: the toxicology database contains acceptable guideline developmental and reproduction studies as well as acute and subchronic neurotoxicity studies; there is no quantitative or qualitative evidence of increased susceptibility following *in utero* exposure in the prenatal developmental study in rats. Although there is qualitative evidence of increased susceptibility in the prenatal developmental study in rabbits and quantitative evidence of increased susceptibility in the 2-generation reproduction study in rats, the HIARC did not identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment of Glufosinate ammonium (See Section 6.B. above). The RfDs established are protective of pre-pre/postnatal toxicity following acute and chronic exposures.

The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

**7. Recommendation for a Developmental Neurotoxicity Study**

The HIARC concluded that there is a concern for developmental neurotoxicity resulting from exposure to glufosinate ammonium.

On June 11, 2002, HIARC recommended that a developmental neurotoxicity study in rats be conducted with glufosinate ammonium based on the following considerations:

- Evidence of neurotoxicity such as increases in the incidence of decreased exploratory activity, decreased alertness, decreased startle response and meiosis in the unacceptable subchronic neurotoxicity study in rats (MRID 42768201).
- Evidence of neurotoxicity such as aggressive behavior, piloerection and a high startle response in a 21-day dermal toxicity study in rats (MRID 40645605).
- Evidence of neurotoxicity such as hyperactivity was observed in dams in a developmental toxicity study in rats (MRID 40345610, 073916, 072965).
- Evidence of neurotoxicity (retinol atrophy) in carcinogenicity study in rats (MRID 43864246, 44539501).
- Lack of acceptable acute neurotoxicity study
- Evidence of neurotoxicity such as tono-clonic convulsions in a 28- day inhalation toxicity study in rats (MRID 40345606).

Evidence to the contrary included:

- No evidence of neurotoxicity in rat offspring in the rat multigeneration reproduction study at levels that caused a decrease in the number of viable pups.
- Susceptibility not demonstrated in developmental or reproductive studies.

The HIARC also recommended that this DNT study should include comparative glutamine synthetase activity measurement in the liver, kidneys, and brain of the pups and mothers (HED Doc. No. 0050900).

On March 27, 2003, based on the weight of evidence presented, the HIARC reaffirmed the previous conclusion that a developmental neurotoxicity (DNT) study with comparative glutamine synthetase activity measurement conducted with glufosinate ammonium in rats is required. HIARC also determined that a 10X database uncertainty factor ($UF_{DB}$) is needed to account for the lack of this study since the available data provide no basis to support reduction or removal of the default 10X factor. The following points were considered in this determination:

- It is assumed that the DNT study (with comparative glutamine synthetase activity measurements) will be conducted at dose levels similar to those used in the rat reproduction study with glufosinate ammonium (2.0, 6.0, and 18.0 mg/kg/day) wherein the offspring NOAEL / LOAEL was 6.0 / 18.0 mg/kg/day, respectively.
- It is likely that the results of the DNT study (with comparative glutamine synthetase activity measurements) could impact the current selected regulatory doses since the NOAELs used for risk assessment endpoints (e.g., 6.3 mg/kg/day for acute and 6.0 mg/kg/day for chronic) are approximately the same order of magnitude as the offspring NOAEL in the rat reproduction study conducted with gluphosinate ammonium (6.0 mg/kg/day).

Therefore, a $UF_{DB}$ of 10X will be applied to all exposure scenarios to account for the lack of the DNT study with comparative glutamine synthetase activity measurement required for glufosinate ammonium.

## HAZARD IDENTIFICATION

### 1. Acute Reference Dose (aRfD) - (Female 13-50 only)

Study Selected: Developmental Toxicity–Rabbit § 83-3b

MRID No.: 40345611, 41144703

EXECITIVE SUMMARY: In a developmental toxicity study (MRID 40345611, 41144703), groups of 15 pregnant female Himalayan rabbits were administered by gavage HOE 039866 at doses of 0., 2.0, 6.3 or 20.0 mg/kg/day from days seven to 19 of pregnancy.

There was a decrease in body weight (6 - 8%, $p \leq 0.05$), body weight gain (37%, $p \leq 0.05$) and food consumption (39%, $p \leq 0.05$) in 20 mg/kg dams. A drop in food consumption (15%, $p \leq 0.05$) was

also seen at 6.3 mg/kg. In the 20 mg/kg group, there were increased kidney weights (11%, $p \leq 0.05$) in the dams. Also at 20 mg/kg/day there was an increase in the number of dead fetuses/litter (0.55/litter vs. 0.00/litter in controls, reported as outside the normal range") and a 4% decrease in mean fetal body weight, also reported as "outside the normal range". Increased incidence of incomplete or absent ossification of skeletal bones in fetuses were observed in the 6.3 and 20.0 mg/kg groups (3 fetuses in 2 litters at 6.3 mg/kg, 9 fetuses in 4 litters at 20 mg/kg. Statistical analysis was not reported). On June 4, 2002, the HIARC considered that the statistically significant increased kidney weights seen at 20 mg/kg/day (HDT) as an adaptive response and not an adverse effect.

Based on the findings presented in this report, the **NOAEL for maternal toxicity was 6.3 mg/kg/day. The LOAEL is 20.0 mg/kg/day based on reduced food consumption, body weight and weight gains. The developmental NOAEL was 6.3 mg/kg/day based on decreased body weights and fetal death seen at 20 mg/kg/day.**

This study is **classified as Acceptable (Guideline)** and meets the requirements for a developmental toxicity study (83-3b) in the rabbit.

Dose and Endpoint for Establishing aRfD: The developmental NOAEL of 6.3 mg/kg/day based on reduced fetal body weights and increased fetal death seen at 20 mg/kg/day (LOAEL).

Uncertainty Factor (UF): 1000; this includes 10x for inter-species extrapolation, 10x for intra-species variation and a 10x data base uncertainty factor for the lack of a study that measures glutamine synthetase in the young and adult animals.

Comments about Study/Endpoint/Uncertainty Factor: The fetal effects are presumed to occur after a single dose. The in utero effects observed are applicable only to the females 13-50 subgroup, and not the general population.

**Acute RfD** (aRfD ) = $\frac{\text{6.3 mg/kg (NOAEL)}}{\text{1000 (UF)}}$ = **0.0063 mg/kg**

**2. Acute Reference Dose (aRfD)** - General Population (including infants and children)

Study Selected: None § N/A

MRID No.: N/A

EXECUTIVE SUMMARY: N/A

Dose and Endpoint for Establishing aRfD: N/A

Uncertainty Factor (UF): N/A

Comments about Study/Endpoint/Uncertainty Factor: An endpoint attributable to a single exposure was not available from the toxicity studies including developmental studies.

**3. Chronic Reference Dose (cRfD)**

Study Selected: A weight-of-evidence approach was used from thee studies; (1) Two-year chronic toxicity/carcinogenicity study in rats, (2) 13-Week oral feeding study in rats (range finding study), and (3) Chronic feeding study in dogs.

MRID No.: 40345607, 41147701 (Two-year chronic toxicity/carcinogenicity study in rats)
45179103 [13-Week oral feeding study in rats (range finding study)]
40345608 (Chronic feeding study in dogs)

EXECUTIVE SUMMARY:

(1). Two-year chronic toxicity/carcinogenicity study in rats

In a combined chronic toxicity/oncogenicity study (MRID 40345607, 41144701) glufosinate ammonium technical (95.3% a.i.) was administered to 50 Wistar rats/sex/dose in the diet for 30 months (carcinogenicity portion) at dose levels of 0, 40, 140, or 500 ppm (mean compound intake in males was 0, 2.1, 6.8, and 24.4 mg/kg/day and for females was 0, 2.4, 8.2 and 28.7 mg/kg/day, respectively). In addition 20 rats/sex/dose were treated for 24 months (chronic portion), and 10 rats/sex/dose were treated for 12 months (interim sacrifice).

There was increased mortality ($p \leq 0.05$) in females at 140 and 500 ppm (30, 46, 54, and 58% deaths at 130 weeks f controls to high dose). The cause of most deaths was reported to be incidental. On June 4, 2002, the HIARC considered that the female deaths were incidental considering the length of the study (130 weeks). Increased kidney glutamine synthetase activity ($p \leq 0.05$) was observed in all treated females and in mid-and high-dose males. Increased absolute and relative kidney weights ($p \leq 0.05$) were observed in mid- and high-dose males, and in all treated females (not a strong dose relation); also, increased kidney to brain weight ratio was observed in males at these dose levels. There was an 11 % inhibition of brain glutamine synthetase in 500 ppm females (male values could not be determined). On June 4, 2002, the HIARC considered that the increased glutamine synthetase activity, increased kidney weight and kidney/brain weight as an adaptive response and not an adverse effect. **The LOAEL is 500 ppm (24.4 mg/kg/day) based on inhibition of brain glutamine synthetase in females at 130 weeks. The NOAEL is 140 ppm (6.8 mg/kg/day).**

**There was no clear demonstration of increased tumor incidence following exposure to glufosinate ammonium.** Dosing was considered adequate in females based on inhibition on brain glutamine synthetase, inadequate in males.

This study is classified as acceptable (guideline), and satisfies the guideline requirement for a chronic toxicity study **(83-1a)** in rats. This study is classified as Acceptable and satisfies the guideline requirement for a cancer study **(83-2a)** in female rats. It is acceptable (guideline) only when considered in combination with the two year cancer data for male rats).

(2) 13-Week oral feeding study in rats (range finding study)

In this study (MRID 45179103), groups of 10 male Wistar rats were fed diets for 6, 13, 20, or 90 days containing 100 or 1000 ppm GA (glufosinate-ammonium; technical concentrate, 50.2% w/w a.i., Lot No. C01284032) or 1000 or 10,000 ppm NAG (N-acetyl-L-glufosinate disodium, 33.8% w/w a.i., Lot No. 2+3/Fassl-4/17714). In addition, groups of 10 rats were treated for 90 days with the test materials followed by a 30-day recovery period. These doses were equivalent to 6.2-8.8 mg/kg/day and 64-90 mg/kg/day for rats receiving 100 or 1000 ppm GA and 65-90 mg/kg/day and 657-935 mg/kg/day for rats receiving 1000 ppm or 10,000 ppm NAG, respectively. At the end of each treatment regimen, the rats were sacrificed, the kidneys, liver and brain removed and weighed, and the glutamine synthetase (GS) activity in these tissues determined.

There were no premature deaths, clinical signs, or treatment-related effects on body weight, body weight gain, or food consumption. Also, treatment had little or no effect on absolute or relative liver or brain weights of the rats; however, the absolute and relative kidney weights of rats treated with the test materials for 6, 13, and 20 days were increased 10 - 20%. In contrast, absolute and relative kidney weights were not increased after 90 days with either test material.

Both GA and NAG inhibited GS activity in the liver and kidney ~30-50 % at their lowest doses and ~40-60% at their highest respective doses. Of the two, the inhibitory effects of GA were approximately 10 times greater than of its metabolite, NAG. Only GA was capable of inhibiting brain GS activity, and only to a minor extent. Extended exposure (90 days) had no biological or additional effects other than the inhibition of GS activity. Both the liver and kidney GS activity recovered on cessation of treatment.

This study demonstrated that GA and its metabolite NAG inhibit the activity of GS, an enzyme responsible for the removal of free ammonia from tissues via the conversion of glutamate to glutamine. The study also showed that GA was approximately 10 times more potent as an inhibitor than NAG. For mammalian systems, however, these findings have little toxicological relevance as numerous metabolic pathways are available for the removal of cytotoxic ammonia.

On June 4, 2002, the HIARC considered that the inhibition of glutamine synthetase in the liver and kidney, and increased in absolute and relative kidney weights as an adaptive response and not an adverse effect.

**The LOAEL for glufosinate-ammonium was 1000 ppm (64-90 mg/kg/day) based on glutamate synthetase inhibition in the brains of males. The NOAEL for glufosinate-ammonium was 100 ppm in the diet (6.2-8.8 mg/kg/day) in males.**

**The LOAEL for N-acetyl-L-glufosinate disodium was 10,000 ppm (657-935 mg/kg/day) based on glutamate synthetase inhibition in the brains of males. The NOAEL for N-acetyl-L-glufosinate disodium was 1000 ppm (65-90 mg/kg/day).**

This study is classified as **Acceptable/Nonguideline** and satisfies its intended purpose of determining the effects of GA and NAG on GS activity.

(3) Chronic feeding study in dogs

In a chronic feeding study (MRID 40345608) HOE 039866 technical was fed to male and female beagle dogs for 12 months in the diet at levels of 2.0, 5.0 or 8.5 mg/kg/day.

There were no overt signs of toxicity or dose-related effects on body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalyses or organ weights. Two dogs receiving 8.5 mg/kg/day died during the study as a result of heart and circulatory system failure from rapid diet consumption and necrotizing aspiration pneumonia. Electrocardiogram results of dosed males and females indicated a dose-related decrease in heart rate at six months; heart rates of dosed animals at 12 months were considered to be normal. **The NOAEL is 5.0 mg/kg/day, the LOAEL is 8.5 mg/kg/day based on mortality (week 2) and alterations in the electrocardiogram at 6 months.**

This chronic toxicity study is classified as **Core Minimum** (83-1b). It satisfies the guideline requirement for a chronic oral study (83-1b) in dogs.

Dose and Endpoint for Establishing cRfD: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Uncertainty Factor(s): 1000; this includes 10x for inter-species extrapolation, 10x for intra-species variation and a 10x data base uncertainty factor for the lack of a study that measures glutamine synthetase in the young and adult animals.

Comments about Study/Endpoint/Uncertainty Factor: The brain glutamine synthetase enzyme inhibition and alterations in electrocardiogram were the most sensitive endpoints following long-term exposure.

**Chronic RfD** = $\frac{6.0 \text{ mg/kg/day (NOAEL)}}{1000 \text{ (UF)}}$ = 0.006 **mg/kg/day**

**4. Incidental Oral Exposure: Short-Term (1-30 days)**

Study Selected: Developmental Toxicity–Rabbit § 83-3b

MRID No.: 40345611, 41144703

EXECUTIVE SUMMARY: See under "Acute Reference Dose (aRfD)-females 13-50 only"

Dose and Endpoint for Risk Assessment: Maternal NOAEL of 6.3 mg/kg/day based on reduced food consumption, body weight and weight gains seen at 20 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This endpoint is appropriate for the population (infants and children) and duration (short-term) of concern.

**5. Incidental Oral Exposure: Intermediate-Term (1 - 6 Months)**

Study Selected: See under "Chronic Reference Dose (cRfD)"

MRID No.: See under "Chronic Reference Dose (cRfD)"

EXECUTIVE SUMMARY: See under "Chronic Reference Dose (cRfD)"

Dose and Endpoint for Risk Assessment: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Comments about Study/Endpoint: This endpoint is appropriate for the population (infants and children) and duration (intermediate-term) of concern.

**6. Dermal Absorption**

Study Selected: Pharmacokinetics with dermal application in rat **§85-2**

MRID No.: 40345620

EXECUTIVE SUMMARY: Groups of male Wistar rats (28/dose level) were dermally administered radioactive HOE 039866 (glufosinate ammonium) at levels of 0.1, 1.0 or 10.0 mg/rat on 6 $cm^2$ of shaved skin. Four rats/dose were exposed for 0.5, 1, 2, 4, 10, 24 or 168 hrs. The quantity of radioactivity in feces, urine and various tissues was measured.

The results indicate that at the low dose (0.1 mg) 42.5 to 50.8% of the applied radioactivity was absorbed whereas at the high dose (10 mg) 26% was absorbed. After removal and washing of the treated skin a substantial amount of the radioactivity still remained in the skin, and it was gradually absorbed and eliminated. Radioactivity was found in both feces and urine samples, but the majority of HOE 039866 was eliminated in the urine. In all organs/tissues examined, radioactivity was found to reach a maximum level either at four or 10 hr after exposure. Subsequently, the radioactivity dropped rapidly. The amount of radioactivity found in the brain was very minimal relative to that of kidneys and liver.

This study is classified as ACCEPTABLE (Guideline), and satisfies the guideline requirements for a dermal penetration study (85-2).

Dermal Absorption Factor: 50%. Percentage dermal absorption is based on the range of 42.5% to 50.8% of radioactivity absorbed at 0.10 mg/kg.

**7. Dermal Exposure: Short-Term (1- 30 days) Exposure**

Study Selected: See under "Acute Reference Dose (aRfD)-females 13-50 only" § 83-3b

MRID No.: See under "Acute Reference Dose (aRfD)-females 13-50 only"

EXECUTIVE SUMMARY: See under "Acute Reference Dose (aRfD)-females 13-50 only"

Dose and Endpoint for Risk Assessment: The NOAEL (maternal and developmental) of 6.3 mg/kg/day based on reduced food consumption, body weight, weight gains, reduced fetal body weight, and increased fetal mortality seen at 20 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The HIARC also evaluated 21-dermal toxicity study in rats for this risk assessment. The dermal NOAEL of 100 mg/kg/day in a dermal toxicity study in rats is not protective of the effects seen in the oral developmental toxicity study in rabbits. In addition, the dermal study did not measure glutamine synthetase activity and developmental effects are not evaluated. Therefore, the HIARC selected an oral study (chosen) for short-term dermal risk assessment. A dermal absorption factor of 50% should be used to extrapolate oral dose to dermal equivalent dose for the dermal risk assessment. Since a NOAEL was selected from developmental toxicity study, a 60 kg body weight should be used in the calculating the human equivalent dose.

**8. Dermal Exposure: Intermediate-Term (1 - 6 Months)**

Study Selected: See under "Chronic Reference Dose (cRfD)"

MRID No.: See under "Chronic Reference Dose (cRfD)"

EXECUTIVE SUMMARY: See under "Chronic Reference Dose (cRfD)"

Dose and Endpoint for Risk Assessment: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Comments about Study/Endpoint: A dermal absorption factor of 50% should be used to extrapolate oral dose to dermal equivalent dose for the dermal risk assessment. Since a NOAEL was selected from developmental toxicity study, a 60 kg body weight should be used in the calculating the human equivalent dose.

**9. Dermal Exposure Long-Term (> 6 Months)**

Study Selected: See under "Chronic Reference Dose (cRfD)"

MRID No.: See under "Chronic Reference Dose (cRfD)"

EXECUTIVE SUMMARY: See under "Chronic Reference Dose (cRfD)"

Dose and Endpoint for Risk Assessment: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Comments about Study/Endpoint: A dermal absorption factor of 50% should be used to extrapolate oral dose to dermal equivalent dose for the dermal risk assessment. Since a NOAEL

was selected from developmental toxicity study, a 60 kg body weight should be used in the calculating the human equivalent dose.

**10. Inhalation Exposure: Short -Term (1- 30 days)**

Study Selected: See under "Acute Reference Dose (aRfD)-females 13-50 only"

MRID No.: See under "Acute Reference Dose (aRfD)-females 13-50 only"

EXECUTIVE SUMMARY: See under "Acute Reference Dose (aRfD)-females 13-50 only"

Dose/Endpoint for Risk Assessment: The NOAEL (maternal and developmental) of 6.3 mg/kg/day based on reduced food consumption, body weight, weight gains, reduced fetal body weight, and increased fetal mortality seen at 20 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The HIARC evaluated the suitability of 28-day inhalation toxicity study in rats with Glufosinate ammonium (MRID 40345606) for this risk assessment. In this study, groups of Wistar rats were exposed to 0, 8, 20, or 46 mg/m$^3$ of glufosinate ammonium for 28 days over a period of 40 days. The NOAEL in this study was 8 mg/m$^3$ (converted to 2.2 mg/kg/day) based on clinical signs (tono-clonic convulsions, staggering gait etc.), decrease in thromboplastin time seen at 20 and 46 mg/m$^3$ (converted to 5.5 and 12.6 mg/kg/day). The HIARC concluded that this study is unsuitable for risk assessment because the particle size is too large, therefore, it decreases the confidence in the study LOAEL/NOAEL. Additionally, the critical effect, brain glutamine synthetase activity, was not measured. Although this study can not be used for risk assessment, the study indicates a high concern for exposure via the inhalation route since it demonstrates a lower NOAEL than those established in the oral studies, indicating that animals are more sensitive to effects by the inhalation route of exposure. The inhalation NOAEL of approximately 2.2 mg/kg/day is about three times lower than the oral NOAELs (6 mg/kg/day) used for end points selected for risk assessments. An additional 10x uncertainty factor is needed for inhalation exposure scenarios to account for this concern.

An inhalation absorption factor of 100% should be used to extrapolate oral dose to inhalation equivalent dose for the inhalation risk assessment. Since a NOAEL was selected from developmental toxicity study, a 60 kg body weight should be used in the calculating the human equivalent dose.

**11. Inhalation Exposure: Intermediate-Term (1- 6Months)**

Study Selected: See under "Chronic Reference Dose (cRfD)"

MRID No.: See under "Chronic Reference Dose (cRfD)"

EXECUTIVE SUMMARY: See under "Chronic Reference Dose (cRfD)"

Dose/Endpoint for Risk Assessment: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Comments about Study/Endpoint: See under "Inhalation Exposure: Short-term, Comments about Study/Endpoint".

**12. Inhalation Exposure: Long-Term (> 6 Months)**

Study Selected: See under "Chronic Reference Dose (cRfD)"

MRID No.: See under "Chronic Reference Dose (cRfD)"

EXECUTIVE SUMMARY: See under "Chronic Reference Dose (cRfD)"

Dose/Endpoint for Risk Assessment: The NOAEL of 6.0 mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram.

Comments about Study/Endpoint: See under "Inhalation Exposure: Short-term, Comments about Study/Endpoint".

**13. Margins of Exposure**

Summary of target Margins of Exposure (MOEs) for risk assessment.

| Route / Duration | Short-Term (1-30 Days) | Intermediate-Term (1 - 6 Months) | Long-Term (> 6 Months) |
|---|---|---|---|
| **Occupational (Worker) Exposure** | | | |
| **Dermal** | 100 | 100 | 100 |
| **Inhalation** | 1000 | 1000 | 1000 |
| **Residential (Non-Dietary) Exposure** | | | |
| **Oral** | 1000 | 1000 | NA |
| **Dermal** | 1000 | 1000 | 1000 |
| **Inhalation** | 3000 | 3000 | 3000 |

**For Occupational exposure: Dermal (All durations), a MOE of 100 is required.** This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation).

**For Occupational exposure: Inhalation (All durations), a MOE of 1000 is required.** This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and

10X for interspecies variation) and an additional uncertainty factor of 10X required due to high concern that exposure via the inhalation route is more sensitive compared to the oral route.

**For Residential exposure: Incidental Oral (Short- and Intermediate-term) and Dermal (All Durations), a MOE of 1000 is required.** This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation) and an additional 10X for the lack of a study that measures glutamine synthetase in the young and adult animals.

**For Residential exposure: Inhalation (All durations), a MOE of 3000 is required.** This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation), an additional 10X for the lack of a study that measures glutamine synthetase in the young and adult animals, and an additional uncertainty factor of 10X required due to high concern that exposure via the inhalation route is more sensitive compared to the oral route. Agency policy limits the total Uncertainty Factor applied for any particular chemical to no more than 3,000 (*A Review of the Reference Dose and Reference Concentration Processes;* EPA/630/P-02/022F; December 2002).

**14. Recommendation for Aggregate Exposure Risk Assessments**

As per FQPA, 1996, when there are potential residential exposures to the pesticide, aggregate risk assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. The toxicity endpoints selected for these routes of exposure may be aggregated as follows: for acute aggregate risk assessment, combine the high end values for both food and water exposure estimates and compare it to the acute RfD.

Short-, intermediate-, and long-term aggregate exposure risk assessment, dermal and inhalation can be combined due to same toxicity end points.

## CLASSIFICATION OF CARCINOGENIC POTENTIAL

**1. Combined Chronic Toxicity/Carcinogenicity Study in Rats**

MRID No. 40345607, 41144701

EXECUTIVE SUMMARY: In a combined chronic toxicity/oncogenicity study (MRID 40345607, 41144701) glufosinate ammonium technical (95.3% a.i.) was administered to 50 Wistar rats/sex/dose in the diet for 30 months (carcinogenicity portion) at dose levels of 0, 40, 140, or 500 ppm (mean compound intake in males was 0, 2.1, 6.8, and 24.4 mg/kg/day and for females was 0, 2.4, 8.2 and 28.7 mg/kg/day, respectively). In addition 20 rats/sex/dose were treated for 24 months (chronic portion), and 10 rats/sex/dose were treated for 12 months (interim sacrifice).

There was increased mortality ($p \leq 0.05$) in females at 140 and 500 ppm (30, 46, 54, and 58%

deaths at 130 weeks f controls to high dose). The cause of most deaths was reported to be incidental. On June 4, 2002, the HIARC considered that the female deaths were incidental considering the length of the study (130 weeks). Increased kidney glutamine synthetase activity ($p \leq 0.05$) was observed in all treated females and in mid-and high-dose males. Increased absolute and relative kidney weights ($p \leq 0.05$) were observed in mid- and high-dose males, and in all treated females (not a strong dose relation); also, increased kidney to brain weight ratio was observed in males at these dose levels. There was an 11 % inhibition of brain glutamine synthetase in 500 ppm females (male values could not be determined). On June 4, 2002, the HIARC considered that the increased glutamine synthetase activity, increased kidney weight and kidney/brain weight as an adaptive response and not an adverse effect. **The LOAEL is 500 ppm (24.4 mg/kg/day) based on inhibition of brain glutamine synthetase in females at 130 weeks. The NOAEL is 140 ppm (6.8 mg/kg/day).**

**There was no clear demonstration of increased tumor incidence following exposure to glufosinate ammonium.** Dosing was considered adequate in females based on inhibition on brain glutamine synthetase, inadequate in males.

This study is classified as acceptable (guideline), and satisfies the guideline requirement for a chronic toxicity study **(83-1a)** in rats. This study is classified as Acceptable and satisfies the guideline requirement for a cancer study **(83-2a)** in female rats. It is acceptable (guideline) only when considered in combination with the two year cancer data for male rats).

Discussion of Tumor Data There was no clear demonstration of increased tumor incidence following exposure to glufosinate ammonium.

Adequacy of the Dose Levels Tested Dosing levels were considered adequate in females based on mortality and inhibition on brain glutamine synthetase at 130 weeks.

**2. Carcinogenicity Study in Rats**

MRID No. 43864246, 44539501

EXECUTIVE SUMMARY: In a rat oncogenicity study (MRID 44539501), glufosinate-ammonium (95.2-96.0% a.i.) was administered to Wistar rats (60/sex/group) for up to 24 months at 0, 1000, 5000, or 10000 ppm (equivalent to 0, 45.4, 228.9, or 466.3 mg/kg/day in males and 0, 57.1, 281.5, or 579.3 mg/kg/day in females).

Survival, clinical signs, body weights, food consumption, differential leukocyte counts, and gross findings for both sexes at all doses were unaffected by treatment with glufosinate-ammonium. Chronic toxicity was characterized by an increase in the incidence of retinal atrophy in treated rats. Retinal atrophy, defined as partial or complete loss of the outer nuclear layer of one or both eyes, was increased in high-dose males (↑200%, $p<0.05$) and females (↑867, $p<0.01$), and mid-dose females (↑533%, $p<0.01$). Absolute and relative (to body) kidney weights compared to concurrent controls were increased ($p<0.05$ or $<0.01$) in the treated animals (absolute, ↑13-27%;

relative, ↑13-35%). Relative kidney (to brain) weights were also increased in the mid- (↑24%, $p<0.05$) and high-dose males (↑25%, $p<0.05$) and all treated female groups (↑15-22%, $p<0.01$). However, no corroborative macroscopic or histopathological data to indicate an adverse effect on the kidneys were observed. The incidence of renal corticomedullary mineralization was decreased ($p<0.05$ or $<0.01$) in all treated females (↓35-53%). In addition, renal caliceal mineralization was decreased ($p<0.05$) in females in the mid- (↓38%) and high-dose groups (↓46%). **The LOAEL for chronic toxicity is 5000 ppm (equivalent to 228.9 mg/kg/day for male rats and 281.5 mg/kg/day for females), based on increased incidences of retinal atrophy. The chronic NOAEL is 1000 ppm.**

**Under the conditions of this study, there was no evidence of carcinogenic potential.**

Dosing was considered adequate based on increased incidences of retinal atrophy.

The oncogenicity study in the rat is determined to be **acceptable (§83-2(a))** and does satisfy the guideline requirement for an oncogenicity study in rats.

Discussion of Tumor Data Under the conditions of this study, there was no evidence of carcinogenic potential.

Adequacy of the Dose Levels Tested Dosing was considered adequate based on increased incidences of retinal atrophy.

**3. Carcinogenicity Study in Mice**

MRID No. 40345609, 41144702

EXECUTIVE SUMMARY: In an oncogenicity study (MRID 40345609, 41144702), HOE 039866 (glufosinate ammonium) was administered to 50 NMRI mice/sex/dose in the diet at dose levels of 0, 80, 160 (males only) or 320 (females only) ppm for 104 weeks. Dose levels corresponded to 0 2.83, 10.82, 22.60 mg/kg/day in males and 0 4.23, 16.19, 66.96 mg/kg/day in females.

There was a dose related increase in mortality 160 ppm males; consistent and sometimes statistically significant decreases in body weight in 160 ppm males of the interim sacrifice animals; statistically significant increases in blood glucose levels in both high-dose males and females at 52 weeks; decreases in albumin and total protein in high-dose females at 52 weeks, decreases in GSH and slight increases in GSSG in whole blood of mid- and high-dose males and also in total GSH and GSSG levels in whole blood of high-dose males; decreases in absolute liver weight and ln liver/body weight ratios in all treated females; and increased incidences of cystic thyroid follicles and of chronic nephropathy in all treated animals. The increase in liver weights in all treated females, and the increases in the incidence of thyroid cystic follicles and chronic nephropathy in all dose males were determined (via subsequent submission of additional data (MRID 4114702) to be NOT treatment related. **The NOAEL for systemic toxicity is 80**

**ppm (10.82 / 16.19 mg/kg/day in M/F), and the LOAEL is 160 /320ppm (22.60 / 63.96 mg/kg/day in M/F), based on increased mortality in males, increased glucose levels in males and females, and consistent changes in glutathione levels in males.**

**No increase in tumor incidence was found in any treatment group.**

This oncogenicity study is classified as acceptable, and satisfies the guideline requirement for a and carcinogenicity study (83-2b) in mice.

Discussion of Tumor Data Under the conditions of this study, there was no evidence of carcinogenic potential in any treatment group.

Adequacy of the Dose Levels Tested Dosing was considered adequate based on increased mortality in males, increased glucose levels in males and females, and consistent changes in glutathione levels in males.

**4. Classification of Carcinogenic Potential** Based on the lack of mutagenic potential as assessed in a battery of mutagenicity assays, and the absence of treatment-related tumors in rats and mice at dose levels adequate for assessment, the HIARC has determined that glufosinate ammonium be classified as a **not likely** carcinogen (HED Doc. No. 013385).

## MUTAGENICITY

The HIARC concluded that there is not a concern for mutagenicity resulting from exposure to Glufosinate ammonium.

### 84-2 Unscheduled DNA Synthesis

**Executive Summary**: In an unscheduled DNA synthesis assay (MRID 40345614), primary rat hepatocyte cultures were exposed to HOE 039886 in deionized water at 15 concentrations ranging from 0.1 to 5240 µg/mL for 18 - 19 hours.

HOE 039866 was tested up to cytotoxic concentrations as evidenced by decreased survival rate as low as 34% **There was no evidence that unscheduled DNA synthesis was induced by the test material.**

This study is classified as **acceptable.** It satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

### 84-2 DNA Damage/Repair in bacteria

**Executive Summary**: In a DNA damage/repair assay ()MRID 072962), glufosinate ammonium was exposed overnight to B. subtilis that lacks the capacity for repair (H45) at concentrations of

0, 50, 100, 500, 1000, 5000 or 10,000 µg/plate. Glufosinate ammonium was also exposed, at the same dose levels, to an isogenic sister strain which has the capacity for DNA repair (H17).

Under the conditions of the study, no difference in the inhibition of growth between these two strains was noted at any of the doses tested. Since the test measures the inhibition of growth in response to the test article, the requirement that chemicals be tested to the limits of cytotoxicity was satisfied. The positive controls, 2-(2-furyl)-3-(5-nitro-2-furyl)acrlamide (AF-2), caused a differential growth inhibition, whereas the negative controls (NaOH, HCL, and Kanamycin) produced no significant difference in growth inhibition. The test system was therefore sensitive to agents that damage DNA. **Under the conditions of the test, the test article failed to cause damage to DNA that could be detected by this repair assay.**

This study is classified as **acceptable**. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (DNA damage & repair) study..

**84-2 Gene mutation assay in Salmonella typhimurium strains**

**Executive Summary**: In a bacterial cell gene reverse mutation assay (MRID 072962) Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were exposed to glufosinate ammonium (92.1% a.i.) at concentrations of 0, 5, 10, 50, 100, 500, and 1000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix).

No increases in mutation frequencies, with or without metabolic activation, were noted in any of the test strains at any of the doses tested. Virtually total inhibition of growth was noted in all strains at the highest dose, 1000 µg/plate. Therefore, the requirement that chemicals be tested to the limits of cytotoxicity was satisfied. The positive controls, 2-aminoanthracene, AF-2, 1-ethyl-2-nitro-3-nitroso-guanidine, 9-amino-acridine, and 2-nitro-fluorine, induced the appropriate responses. Therefore the test systems were sensitive to agents that induce gene mutation. Under the conditions of the test, glufosinate- ammonium failed to cause reverse mutations in bacteria with and without metabolic activation

This study is classified as **acceptable**. It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity (bacteria reverse gene mutation) data.

**84-2 Mouse Lymphoma Forward Mutation Assay**

**Executive Summary**: In a mouse lymphoma L5179Y forward mutation assay(MRID 40345616.) HOE 039866 was tested at seven nonactivated doses of 50 to 5000 µg/mL or at six S9-activated doses of 300 to 5000 µg/mL.

HOE 39866 did not increase the mutation frequency at the thymidine kinase locus. The solvent controls gave acceptable values and the positive controls ethylmethanesulfonate (nonactivated) and 3-methylcholanthrene (S9-activated) provided evidence that the assay had adequate sensitivity for detecting mutagenicity.

This study is classified as **acceptable**. It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity (mouse lymphoma forward mutation) data.

### 84-2 Mouse Micronucleus Assay

**Executive Summary**: In a mouse micronucleus assay (MRID 41144704.) 13 groups of mice (5/sex/dose) received a single administration of HOE 039866 at dose levels of 100, 200, and 350 mg/kg by gavage. A positive control group received 50 mg/kg of cyclophosphamide. After dosing, the animals were sacrificed at 24, 48, and 72 hrs., and the erythrocytes from the bone marrows were sampled at these times. The results indicated the test agent had no effect on micronucleus formation. This observation was consistent with that of a previous in vivo micronucleus assay (HED Document Nos. 004403, 004928, 006936).

This study is classified as **acceptable**. It satisfies the requirement for FIFRA Test Guideline 84-2 for in vivo mutagenicity (mouse micronucleus) data.

## HAZARD CHARACTERIZATION

Glufosinate ammonium (also referred to as DL-glufosinate ammonium or HOE 039866 ) is toxicity category III for acute oral, dermal and inhalation toxicities. It is toxicity category II for eye irritation. It is not a dermal irritant nor is it a dermal sensitizer. For subchronic toxicity, the primary effects in the mouse were increased liver and kidney weights with increases in serum aspartate amino transferase and alkaline phosphatase. Signs of neurotoxicity were observed in rats in subchronic studies, such as aggressive behavior, piloerection, high startle response, increased incidence of fearfulness.

In the chronic studies in the rat, increased mortality, increased occurrence of retinal atrophy, and inhibition of brain glutamine synthetase were observed, as were increased liver and kidney weights. In the mouse, increased mortality was observed, as was changes in glucose levels consistent with changes in glutathione levels. Increased mortality and EKG alterations were observed in dogs. **There was no evidence of a treatment-related increase in tumors.**

The developmental toxicity study in the rat produced dilated renal pelvis and/or hydroureter in the offspring at levels that produced significant increases in hyperactivity and vaginal bleeding in dams. In the rabbit, decreased fetal body weight and increased mortality were observed at 20 mg/kg/day, while in rabbit dams, decreased food consumption, body weight and body weight gain were observed at 20 mg/kg/day. Since increased pup mortality was observed in the presence of maternal toxicity, **there is evidence of qualitative increased susceptibility in offspring in the rabbit developmental toxicity study.**

The reproductive toxicity study indicated postnatal developmental toxicity at 18.0 mg/kg/day (HDT) in the form of decrease in viable pups. No parental toxicity was seen at HDT. Since developmental effects were observed at dose levels below the parental toxicity, **there is evidence of quantitative increased susceptibility in offspring.**

A **consistent pattern of neurotoxicity was seen** in several studies, including the subchronic, developmental and chronic studies in rats, mice and dogs. In addition to the clinical signs such as hyperactivity, aggressive behavior, tono-clonic convulsion, piloerection, high startle response, retinal atrophy was observed. Changes in glutamine synthetase levels were observed in liver, kidney and brain in rats. The HIARC concluded that the changes in liver and kidney glutamine synthetase activity and changes in liver and kidney weights as an adaptive response and not an adverse effect. The HIARC also concluded that the changes in brain glutamine synthetase activity is of significant concern. It is expected that the requested special studies (see Data Gaps section) will provide the information needed to confirm these conclusions and further characterization of these effects.

There is no concern for mutagenic activity in several studies including: Salmonella E. Coli, *in vitro* mammalian cell gene mutation assays, mammalian cell chromosome aberration assays, *in vivo* mouse bone marrow micronucleus assays, and unscheduled DNA synthesis assays.

A rat metabolism study with dermal application indicated that about 50% of the given radioactivity was absorbed 48 hours after a single dose application. In other metabolism studies, it was shown that over 80% of administered radioactivity is excreted within 24 to 48 hours as the parent compound in the feces and kidneys. In the urine, two metabolites (HOE 061517 and HOE 086486) were identified in minor amounts. In the feces, two additional metabolites (HOE 099730 and HOE 042231) were detected in minor amounts. Highest tissue levels were found in liver, kidney and gonads.

Additional testing was conducted in the major metabolites, known as HOE 061517 and HOE 099730, as well as the L-isomer, known as HOE 058192. These compounds, tested in subchronic rat, mouse and dog studies, and in developmental toxicity studies in rat and rabbit showed a similar profile of toxicity as the parent compound (HOE 039866).

**Mode of Action**: Because the herbicidial mechanism of action of glufosinate-ammonium is inhibition of the enzyme glutamine synthetase and because this enzyme is present in mammalian systems, the action of glufosinate-ammonium on glutamine synthetase in the liver, kidney, and brain of the rat was investigated. Glutamine synthetase facilitates the conversion of glutamate and ammonia to glutamine and is therefore involved in the metabolism of nitrogen and ammonia. In addition, glutamate is a major excitatory neurotransmitter in the nervous system; inhibition of glutamine synthetase has been shown to impair its ability to serve as a neuroprotectant by controlling glutamate concentrations in neurons (Gorovits et al., 1997). More generally in the body, ammonia is buffered for extracellular transport through its interaction with glutamate to form glutamine by glutamine synthetase (Kelly and Stanley, 2001).

Gorovits R, Avidan N, Avisar N, Shaked I, Vardimon L. 1997. Glutamine synthetase protects against neuronal degeneration in injured retinal tissue. Proc Nat. Acad. Sci. 94:7024-7029.

Kelly A, Stanley CA. 2001. Disorders of Glutamate Metabolism. Mental Retard and Devel Disabil Res Reviews 7: 287-295.

## DATA GAPS / REQUIREMENTS

1. Comparative measurements of glutamine synthetase activity (brain, kidney and liver) in young and adult animals.

2. A Developmental Neurotoxicity Study (DNT) in rats (previously required by HIARC).

3. Repeat of Acute Neurotoxicity Study in rats with glufosinate ammonium (only) with adequate dosing as per the guideline. This study should also include measurements of glutamine synthetase activity (brain, kidney and liver).

4. A 28-day inhalation toxicity study in rats with glutamine synthetase activity measurements in brain, kidney, liver and lung).

5. Additional data are required to confirm that liver and kidney changes in the absence of histopathological changes are adaptive response and not an adverse effect. It should include kidney and liver function assays in addition to glutamine synthetase activity measurements and required routine parameters.

## ACUTE TOXICITY

Acute Toxicity of Glufosinate ammonium Technical

| Guideline No. | Study Type | MRID #(S). | Results | Toxicity Category |
|---|---|---|---|---|
| 81-1 | Acute Oral | Accession No. 072962 | $LD_{50}$ = 4010 mg/kg in males<br>$LD_{50}$ = 3030 mg/kg in females | III |
| 81-2 | Acute Dermal | Accession No. 072962 | $LD_{50}$ = >2000 mg/kg in males & females | III |
| 81-3 | Acute Inhalation | Accession No. 073916 | $LC_{50}$ = 4.42 m/L estimated in males & females | III |
| 81-4 | Primary Eye Irritation | Accession No. 072962 | eye irritant, corneal opacity reversible within 72 hours | III |
| 81-5 | Primary Skin Irritation | Accession No. 072962 | not a dermal irritant | IV |
| 81-6 | Dermal Sensitization | Accession No. 072962 | not a dermal sensitizer | N/A |

## VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Summary of Toxicology Endpoint Selection for Glufosinate ammonium

| Exposure Scenario | Dose Used in Risk Assessment, UF | Special FQPA SF* and Level of Concern for Risk Assessment | Study and Toxicological Effects |
|---|---|---|---|
| **Acute Dietary** (Females 13-50) | NOAEL = **6.3**<br>UF = **1000**<br><br>**Acute RfD** = **0.0063** mg/kg/day | FQPA SF = **1X**<br><br>**aPAD** = acute RfD / FQPA SF = **0.0063** mg/kg/day | **[Developmental Toxicity Study in Rabbits]**<br>LOAEL = **[20 ]** mg/kg/day based on **[reduced fetal body weight and increased fetal death ]**. |
| **Acute Dietary** (General Population including infants and children) | Mat. NOAEL =N/A<br>UF =**N/A**<br><br>**Acute RfD** =**N/A** | FQPA SF = N/A<br>**aPAD** = acute RfD / FQPA SF = N/A | No endpoint attributable to a single exposure was identified for the general population, including infants and children. |
| **Chronic Dietary** (All populations) | NOAEL= **6.0**<br>UF = **1000**<br><br>**Chronic RfD** = **0.006** mg/kg/day | 1X<br><br>**cPAD** = chronic RfD / FQPA SF =**0.006** mg/kg/day | **["Weight-of-evidence" approach from several studies]**<br>NOAEL = **[6.0 ]** mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram. |
| **Short-Term** (1-30 days) **Incidental Oral** | Mat. NOAEL= **6.3** mg ai/kg/day | **Residential** MOE = **1000**<br><br>**Occupational** = NA | **[Developmental Toxicity Study in Rabbits]**<br>LOAEL = **[20 ]** mg/kg/day based on **[reduced food consumption, body weight and body weight gain ]**. |
| **Intermediate-Term** (1 - 6 months) **Incidental Oral** | NOAEL= **6.0** mg ai/kg/day | **Residential** MOE = **1000**<br><br>**Occupational** = NA | **["Weight-of-evidence" approach from several studies]**<br>NOAEL = **[6.0 ]** mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram |
| **Short-Term** (1 - 30 days) **Dermal** | Oral NOAEL= **6.3** mg ai/kg/day[a] | **Residential** MOE = **1000**<br><br>**Occupational** MOE = **100** | **[Developmental Toxicity Study in Rabbits]**<br>LOAEL = **[20 ]** mg/kg/day based on **[reduced fetal body weights, increased fetal mortality, reduced food consumption, and decreased body weight and body weight gain ]**. |

| Exposure Scenario | Dose Used in Risk Assessment, UF | Special FQPA SF* and Level of Concern for Risk Assessment | Study and Toxicological Effects |
|---|---|---|---|
| **Intermediate-Term** (1 - 6 months) and **Long-Term Dermal** (>6 months) | Oral NOAEL= **6.0** mg ai/kg/day[a] | **Residential** MOE = **1000**<br><br>**Occupational** MOE = **100** | **["Weight-of-evidence" approach from several studies]** NOAEL = **[6.0 ]** mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram |
| **Short-Term** (1 - 30 days) **Inhalation** | Oral NOAEL= **6.3** mg/kg/day[b] | **Residential** MOE = **3000**<br><br>**Occupational** MOE = **1000** | **[Developmental Toxicity Study in Rabbits]** LOAEL = **[20 ]** mg/kg/day based on **[reduced fetal body weights, increased fetal mortality, reduced food consumption, and decreased body weight and body weight gain ]** |
| **Intermediate-Term** (1 - 6 months) and **Long-Term Inhalation** (>6 months) | Oral NOAEL= 10 mg/kg/day[b] | **Residential** MOE = **3000**<br><br>**Occupational** MOE = **1000** | **["Weight-of-evidence" approach from several studies]** NOAEL = **[6.0 ]** mg/kg/day based on brain glutamine synthetase inhibition and alterations in the electrocardiogram |
| Cancer | **Classification: Not likely to be carcinogen**<br>**Q1* = N/A** | | |

a = Dermal absorption factor: 50%
b = Since oral values were selected 100% inhalation absorption factor (default) value should be used in route-to-route extrapolation/risk assessment.

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

***NOTE:*** The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR NO. 0050727

**MEMORANDUM**

Date: 9-May-2002

Subject: PP#s - 0F06210 (transgenic rice), 0F06140 (transgenic cotton), and 2E06404 (blueberry) - Glufosinate Ammonium in/on Transgenic rice, Transgenic Cotton, and Blueberry. **Health Effects Division (HED) Metabolism Assessment Review Committee (MARC) Decision Document.** DP Barcode D282757. Chemical 128850. Case 292945. Submission S596735.

From: Tom Bloem, Chemist, Registration Action Branch 1 (RAB1)/HED (7509C)
PV Shah, Ph.D., Toxicologist, RAB1/HED (7509C)

Through: Christine Olinger, HED MARC Chair (7509C)
G. Jeffrey Herndon, Branch Senior Scientist, RAB1/HED (7509C)

To: Yan Donovan, HED MARC Executive Secretary (7509C)

**Material Reviewed**

The HED MARC met on 24-April-2002 to evaluate the glufosinate-ammonium plant metabolism studies, livestock metabolism studies, confined rotational crop studies, environmental fate and persistence data, and toxicological data. Tom Bloem, PV Shah, and John Ravenscroft were responsible for data review and preparation of the briefing document (D282473, 22-Apr-2002).

**MARC Members in Attendance:** Christine Olinger, Rick Loranger, Alberto Protzel, Norman Birchfield, David Nixon, Bill Wassell, Leung Cheng, John Doherty, Steve Knizner, and Leonard Keifer.

**MARC Members in Absentia:** Abdallah Khasawinah, Sheila Piper and Yan Donovan

**non-MARC Members in Attendance:** Tom Bloem (RAB1), PV Shah (RAB1), John Ravenscroft (EFED), and Jeffrey Herndon (RAB1)

*Plants:* Metabolism studies conducted with non-transgenic (corn, soybean, apple, and lettuce) and transgenic (corn, soybean, sugar beet, canola, and rice) crops were submitted. The transgenic crops contain phosphiothrion-acetyl-transferase (PAT) which acetylates glufosinate to N-acetyl glufosinate which is not herbicidally.

HOE 061517 was the only metabolite identified in the non-transgenic studies (2-40% total radioactive residue (TRR); only soybean leaf, corn stover, and apples were analyzed). The petitioner demonstrated that 40% of the TRR in non-transgenic corn stover was incorporated into protein, starch, cellulose, and lignin. HOE 039866, HOE 099730, and HOE 061517 were the major residues identified in the transgenic crops (40-98% of the TRR). The petitioner demonstrated that for transgenic crops, surface residues are composed of a nearly equal mixture of the D and L isomers of HOE 039866 (technical glufosinate ammonium is a racemic mixture) while interior residues are composed of almost exclusively D isomer of HOE 039866. This indicates that only the L-isomer of HOE 039866 is acetylated to form HOE 099730.

Based on the metabolism and magnitude of the residue studies, the MARC concluded that the residues of concern in the crops studied, for tolerance expression and risk assessment purposes, are HOE 039866, HOE 099730, and HOE 061517. Since the analytical enforcement method can not distinguish between the D- and L-isomers of HOE 039866 and HOE 099730, the tolerance expression will include both isomers.

*Livestock:* Lactating goat and laying hen metabolism studies conducted at 7x the current maximum theoretical dietary burden (MTDB) were submitted (animals were fed [3,4-$^{14}$C]-HOE-039866). TRRs in muscle and fat from both studies were <0.01 ppm and were not further analyzed. Kidney, liver, and milk from the goat study and egg and liver from the hen study were analyzed with 36-90% of the TRR identified as HOE 039866 and HOE 064619. HOE 099730 was identified as a minor metabolite in both the goat and hen studies (≤5% TRR).

Since the majority of the livestock dietary burden originates from transgenic crops, HOE 099730 will be the primary residue in/on treated feed commodities. HOE 099730 was found as minor metabolite in the livestock metabolism studies indicating that this compound is part of the HOE 039866 metabolic pathway for livestock. Therefore, metabolism studies conducted with HOE 099730 are unnecessary.

Based on the metabolism and feeding studies, the MARC determined that the residues of concern in livestock, for tolerance expression and risk assessment purposes, are HOE 039866, HOE 099730, and HOE 061517. Since the analytical enforcement method can not distinguish between the D- and L- isomers of HOE 039866 and HOE 099730, the tolerance expression will include both isomers.

*Rotational Crops:* A confined rotational crop study has been submitted in which lettuce, radish, and spring wheat were planted 28 and 119 days after the soil was treated with [3,4-$^{14}$C]-HOE-039866 at 0.9 lbs ai/acre (0.8x the maximum rate for registered/proposed crops which are likely to be rotated). All samples planted 28 days after treatment were analyzed. HOE 061517 (5-57% TRR) and HOE 064619 (6-10% TRR) were the only compounds identified (a total of 32-64% of the TRR was identified). Except for the wheat commodities, TRRs were ≤0.02 ppm for the samples planted 120 days after treatment (wheat commodities 0.06-0.15 ppm). A field rotational crop study was submitted in which wheat was planted 73 - 90 days after the soil was treated with glufosinate ammonium at 0.8 lbs ai/acre (0.7x the maximum application rate for registered/proposed crops likely to be rotated). Wheat forage, hay, straw, and grain were harvested at maturity and analyzed for residues of HOE 039866 and HOE 061517 (residues were < limit of quantitation (LOQ); LOQ = 0.05 ppm).

Based on the confined and field rotational crop studies, the MARC determined that the residues of concern in rotational crops, for tolerance expression and risk assessment purposes, are HOE 039866, HOE 061517, and HOE 064619. Since the analytical enforcement method can not distinguish between the D- and L-isomers of HOE 039866, the tolerance expression will include both isomers.

*Drinking Water:* The available environmental fate studies indicate glufosinate ammonium degrades fairly rapidly (half life on soil ranging from 4 to 23 days) and is very mobile. In aerobic soil metabolism studies, glufosinate ammonium was degraded primarily to $CO_2$, HOE 061517 (39-52% of the applied dose), and HOE 064619 (6-18% of the applied dose). The degradate HOE 086486 was observed as a minor degradate (<1.0-5.4% of the applied dose) in aerobic soil metabolism. In addition to the soil metabolism degradates, the degradate HOE 099730 (a.k.a. HOE 085355) was observed in the soil photolysis study (2-15% of the applied dose). From studies on the soil

affinity of the aerobic soil metabolism degradates, both HOE 061517 (Kd=0.7) and HOE 086486 (Kd=0.8) were found to bind weakly to soil and are thus expected to be more mobile than glufosinate ammonium (Kd=1.5). HOE 064619 binds more strongly to soil (Kd =24) than glufosinate ammonium and is expected to be less mobile. Limited persistence information is available on the degradates, but HOE 061517 appears to be relatively stable in soil (half life >120 days).

Based on the environmental fate studies, the MARC concluded that glufosinate ammonium, HOE 061517, HOE 064619, and HOE 099730 are residues of concern in drinking water for purposes of risk assessment. Since HOE 086486 was never present at greater than 5.4% of the dose applied, this degradate was not included as a residue of concern.

*Toxicity of Metabolites:* Glufosinate ammonium (HOE 039866) is a racemic mixture of the D- and L-isomers; only the L-isomer is herbicidally active. The petitioner has submitted acute, subchronic, and developmental toxicity studies for HOE 058192 (L-glufosinate ammonium), HOE 099730, and HOE 061517. Based on a comparison of the $LD_{50}$ of the acute toxicity studies and no observable adverse effect levels (NOAELs) of the subchronic and developmental toxicity studies, HOE 099730 and HOE 061517, in general, are less toxic than HOE 039866. Based on a comparison of the $LD_{50}$ of the acute toxicity studies and NOAELs of the subchronic and developmental toxicity studies, HOE 058192 is more toxic than HOE 039866 (D229929, W. Phang, 24-Jun-1998). Chronic toxicity studies used to select endpoints for long-term dietary exposures are not available for the metabolites.

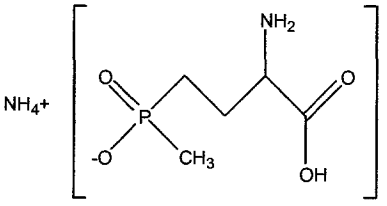
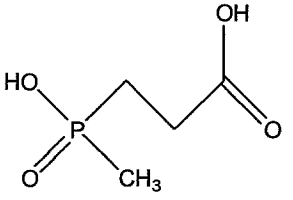
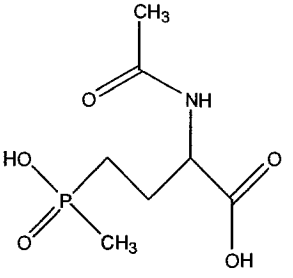
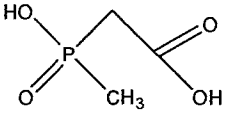
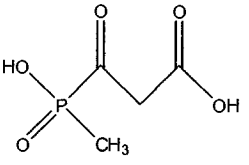
As stated in the previous paragraph, limited toxicity data suggest that HOE 058192 (L isomer of glufosinate ammonium) may exhibit slightly greater toxicity than HOE 039866 (racemic mixture of glufosinate ammonium). HOE 039866 was not found in the non-transgenic metabolism studies and was found at 2-42% of the TRR in transgenic plants (methods did not distinguish between the D and L isomers). Stereochemical separation of the D and L isomers of glufosinate was performed on the rinse and extract of transgenic sugar beet leaf samples. The results showed approximately equal concentrations of both isomers in the rinse. The extract consisted of almost entirely the D isomer. Therefore, exposure to only HOE 058192 is considered unlikely.

Based on the toxicity studies, structure-activity relationships, and/or the identification of the residues of concern in the rat and dog metabolism studies, the MARC concluded that HOE 099730, HOE 061517, and HOE 64619 are not likely to be more toxic than parent. For purposes of risk assessment, all the metabolites of concern should be considered to be equally toxic as HOE 039866.

**Attachment 1:** chemical structures

reference: MARC briefing document - D282473, 22-Apr-2002
cc: T. Bloem (RAB1), J. Miller (RD), F. Griffith (BEAD)
RDI: RAB1 Chemists (9-May-2002)

**Attachment 1:** chemical structures

| Chemical Name | Chemical Structure |
|---|---|
| glufosinate ammonium<br>HOE 039866<br><br>CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt<br><br>technical is a racemic mixture of the D and L isomers |  |
| HOE 061517<br><br>IUPAC name - 3-methylphosphinico-propionic acid<br><br>CAS name - 3- (hydroxymethylphosphinyl)-propionic acid |  |
| HOE 099730<br><br>IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid<br><br>CAS name - L-2-(acetylamino)-4-(hydroxymethyl-phosphinyl)butanoic acid<br><br>common name: L-N-acetyl glufosinate ammonium<br><br>the tolerance expression will include both the D and L isomers |  |
| HOE 064619<br><br>2-methylphosphinico-acetic acid |  |
| HOE 086486 |  |

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|---|---|
| **Chemical:** | **Glufosinate** |
| **PC Code:** | **128850** |
| **HED File Code** | **11000 Chemistry Reviews** |
| **Memo Date:** | **08/21/2003** |
| **File ID:** | **DPD292894** |
| **Accession Number:** | **412-04-0038** |

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**11/12/2003**